Sensory Biology of Sharks, Skates, and Rays

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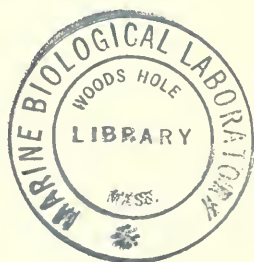# Sensory Biology of Sharks, Skates, and Rays

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1978

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Dedicated to Albert Tester (1908–1974), an outstanding pioneer in the study of the sensory biology of elasmobranchs.

A large lemon shark (*Negaprion brevirostris*) attacks air bubbles at the sea surface, during frenzy behavior elicited by stimulation with an amine and amino acid mixture. For details, consult the article by E. S. Hodgson and R. F. Mathewson, pp. 227–267. (Photo by Edward Hodgson.)

FOREWORD

Sharks and their close relatives have been among the most important and successful inhabitants of the seas for some 200 million years. It is hardly surprising, therefore, that man—a relatively recent invader of marine environments—finds much of importance to be learned from studies of these remarkably adapted animals.

Since many sharks are dominant predators in the sea, their attacks upon man typically elicit sensational reporting. The International Shark Attack File, supported in part by the U.S. Navy and compiled in cooperation with the Smithsonian Institution, is the major source of factual information on that subject and has provided useful guidelines for diminishing the chances of attacks upon humans. Despite the availability of constantly improving rescue methods and more advanced medical attention for personnel in areas where shark attacks are prevalent, even the remote possibility of experiencing such an attack is a powerful psychological factor, often a significant handicap, for those working in such waters. Information about the distribution, biology, and behavior of sharks can help to further increase the odds in man's favor during exposure to such possibilities or whenever encounters with the genuinely dangerous species occur.

The Office of Naval Research takes pride in having supported, through the Oceanic Biology Program of the Ocean Science and Technology Division, many of the research studies reported here. It seems appropriate at this time to assist the further synthesis and distribution of this information through the present volume. It is hoped that the benefits for present science and future research, like the knowledge that the book brings together, will be worldwide.

R. K. GEIGER
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PREFACE

Different areas of science characteristically enjoy "peak" periods, during which curiosity and concern about unsolved problems, availability of research support, and emerging new results and insights combine to yield eras of exceptional productivity. Shark research is enjoying such a peak phase at the present time. This makes it an especially exciting, as well as a useful, time to take stock of what is known (and what is *not* known) in this area of sensory physiology and animal behavior.

People are interested in sharks, skates, and rays for many reasons. At some point in their studies, virtually all biology students encounter diagrams or preserved specimens of sharks, the anatomical features of which provide a good starting point for understanding the basic body forms of backboned animals, including man. Marine biologists, dealing with living elasmobranchs in the sea, are fascinated by the many and superb adaptations of these animals that account for their long evolutionary success. People whose work or recreation takes them into the sea are concerned about the possibilities of being attacked by sharks; thus they are interested in the work of sensory physiologists and ethologists who attempt to understand the behavioral patterns, with their underlying mechanisms, in predatory species. Most recently, engineers and technologists have recognized that some sense organs of elasmobranchs far exceed the capacities of our human sensory apparatus and therefore might provide clues or prototypes for better instrumentation to assist human probings of the sea.

Two brief examples illustrate the latter types of interest. The olfactory receptors of sharks are highly efficient detectors of waterborn chemicals. In some cases, only a few molecules contacting these chemoreceptors can trigger powerful responses from sharks, well before any analytical methods now used by chemists can signal the presence of the molecules. In an age when man's needs to sample and monitor the chemistry of the seas (and the effects of human activities upon them) are increasing, it is not unreasonable to anticipate that our chemical detecting systems might profit from better understanding of those that already serve the sharks so well.

In other cases, the sharks, skates, and rays have sensing mechanisms that are completely absent in ourselves. Recent research has shown that sharks use bioelectrical cues from their prey to guide close-range attacks. The stingray, for another example, has a sensory mechanism for detecting weak magnetic forces and regularly uses this sense for orienting to the geomagnetic field of the earth. What a convenience such an inborn sense would be to human navigators!

The sense organs are the logical starting point for the investigation of behavior in any animals, as well as for attempts to exploit the special sensory capacities of animals in perfecting new technologies for human purposes. That is why the contributions brought together in this book are particularly significant at this time. They summarize the present "state of the art" in research on some of the best underwater sensing systems ever evolved.

In soliciting and discussing contributions from many scientists, we found general agreement that a wide-ranging coverage, leading toward perspectives on currently unsolved problems, would be most useful. Consequently, a sizeable representation of the behavioral and natural history aspects of elasmobranchs has been included, along with the strictly anatomical and physiological studies. Observations from people with long and extensive fisheries experience, but virtually no previously written records about it, seemed important for their clues to problems that physiologists might well investigate. Studies dealing with the effects of confinement upon the sensory apparatus of sharks also seemed important for future laboratory investigators. It will be readily apparent to those with experience in this field why the title refers to sensory *biology*, rather than strictly sensory *physiology*. We hope this will help to emphasize the many important observations within the sphere of natural history that remain to be investigated on a physiological level.

It is a pleasure to acknowledge our indebtedness to Drs. Charles Woodhouse, Jr., and Bernard Zahuranec of the Oceanic Biology Program of the Office of Naval Research, who originally suggested the book, and to Drs. Ronald Tipper and Eric Shulenberger who have continued to provide ONR's support, without which this volume could not have materialized. Mr. Stanley Smith and Mrs. Sara Curry, of the Editorial Branch of the Naval Research Laboratory, have given us invaluable guidance on editorial matters, and Mrs. Curry has patiently and expertly guided the final preparation of the material for the printers. Mr. DeWitt Darr, Sr., and Mrs. Dolores Robbins, of the Graphic Arts Branch, NRL, have ably and helpfully overseen the layout and production aspects of the volume.

Finally, we welcome this chance to thank again our colleagues who made contributions to the book. In addition to those who wrote chapters, our thanks include others who were generous in enthusiasm and helpful suggestions, but who found themselves with conflicting commitments so that they could not contribute more explicitly. In the end, whatever values this book may have are the result of the sustained efforts of all these people.

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I PERSPECTIVE

SHARKS IN PERSPECTIVE

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INTRODUCTION

Except for occasional flutters of publicity when attacks occurred, sharks were of academic interest to only a few scientists before World War II. Then, when servicemen were victims of air and sea disasters and met sharks in their own environment with tragic consequences for the men, the U.S. Navy gathered a team of experts to produce a solution. An excellent account by Bernard Zahuranec of Shark Chaser, the chemical repellent, and its initial acceptance and subsequent rejection may be found in another section.

A veritable explosion of investigations in shark biology and a mushrooming of knowledge of sharks followed creation of the ONR-sponsored AIBS Shark Research Panel, a product of the New Orleans Shark Conference in April 1958 (Fig. 1). Members of the original panel were Sidney R. Galler, John R. Olive, Leonard P. Schultz, Stewart Springer, and Perry W. Gilbert, chairman. Albert L. Tester and H. David Baldridge became members in 1964 and 1968, respectively. The panel catalyzed and coordinated more

Figure 1 Four distinguished participants in the New Orleans Shark Conference, April 1958. Left to right: Gilbert P. Whitley, Australian Museum, Sydney, Australia; Leonard P. Schultz, Smithsonian Institution, Washington, D.C.; J. L. B. Smith, Rhodes University, Grahamstown, South Africa; Carl L. Hubbs, Scripps Institution of Oceanography, La Jolla, Calif.

than 100 studies of the biology and behavior of sharks in many parts of the world. Besides conducting their own basic research, several panel members (Fig. 2) tested more than 200 chemical compounds, biological products, and physical devices for their deterrent effect on sharks. The results have appeared in many publications (Gilbert 1963, Gilbert et al. 1967). While fulfilling their practical objectives, these tests disclosed new facts about the response patterns and behavior of many species of sharks. These observations, supplemented by a wide range of basic studies of the phylogeny, taxonomy, anatomy, physiology, distribution, migrations and life history, behavior, ecology, pharmacology, endocrinology, and immunology of sharks during the last 15 years, have provided us with a wealth of knowledge of these highly successful vertebrates.

ARE SHARKS UNPREDICTABLE?

Sharks are frequently called unpredictable. While this is true within limits, we now know that many behavioral and metabolic responses of sharks can be predicted. In the western North Atlantic we can predict with considerable accuracy when spiny dogfish (*Squalus acanthias*) will begin their spring
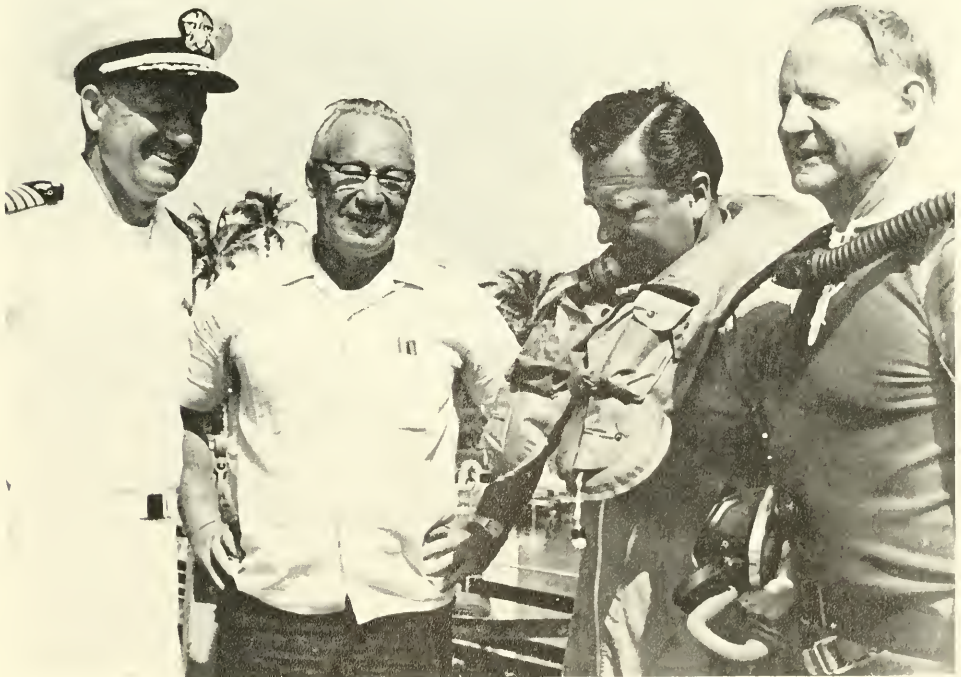

Figure 2 Four participants in 1966 shark deterrent tests at the Lerner Marine Laboratory, Bimini, Bahamas. Left to right: H. David Baldridge, Albert L. Tester, C. Scott Johnson, Perry W. Gilbert. (Photograph by Robert F. Mathewson.)

migration from North Carolina coastal waters and when they will arrive off
the coast of northern Maine and Newfoundland. Likewise, we can predict
the time of their return in the fall (Templeman 1944, Jensen 1966). Olsen
(1954, 1959) has established the migratory movements of the edible
Australian school shark (*Galeorhinus Australis*), which has been a boon
to commercial fishermen.

The movements of several species, including dusky (*Carcharhinus ob-
scurus*), sandbar (*Carcharhinus milberti*), lemon (*Negaprion brevirostris*),
bull (*Carcharhinus leucas*), and nurse sharks (*Ginglymostoma cirratum*),
along the gulf coast of Florida are predictable (Clark and von Schmidt
1965). This knowledge has been of great help to the collecting crew of
the Mote Marine Laboratory in Sarasota, Florida. The time of birth and
mating is also now well known for several species, including nurse sharks
(*Ginglymostoma cirratum*) at Dry Tortugas, Port Jackson sharks (*Hetero-
dontus portusjacksoni*) in Australia (McLaughlin and O'Gower 1971),
and sandbar sharks (*Carcharhinus milberti*) off the east coast of Florida
(Springer 1960, 1967). The gestation periods of certain viviparous sharks
are now well established; one species, *Squalus acanthias*, holds the verte-
brate record—an incredible 20–22 months (Hisaw and Albert 1947, Gilbert
and Heath 1972).

Much has been learned about shark behavior in the past 20 years, and
many of their responses can now be predicted. The agonistic display of
the gray reef shark (*Carcharhinus menisorrah*) (Johnson and Nelson 1973)
warns the diver who encounters it. The attraction of several species of
sharks to low-frequency sounds, simulating those of struggling fish, has
been well established by Evans and Gilbert (1971), Nelson and Johnson
(1972), Brown (1973), and Myrberg et al. (1975), among others. The
orientation patterns of lemon sharks (*Negaprion brevirostris*) and nurse
sharks (*Ginglymostoma cirratum*) to specific chemicals of known dilution
are predictable (Mathewson and Hodgson 1972).

While fresh fish and mammalian blood are moderately strong attractants
to several species, fresh tuna or bonito juice has repeatedly proved even
stronger. Even with such attractants it is often difficult to get captive sharks
to begin feeding. Once one shark in a group has rushed at a bait, however,
others quickly follow and a "feeding frenzy" often develops. Such active
competition for food and other desirable items is not restricted to the
elasmobranch level!

The attraction of many species of sharks by bright or shining objects
is predictable. For this reason, dark, nonreflective colors have been recom-
mended for the submerged portion of shark deterrents such as the Johnson
Shark Screen (Gilbert 1968a) and the Federal Aviation Administration's
infant floatation device (Gilbert 1970). The responses of many species
of sharks to electric fields are well known and predictable, and Kalmijn
(1971) has demonstrated that *Scyllorhinus canicula*, using the ampullae
of Lorenzini as electroreceptors, can locate living prey, even if the prey
lies buried in the sand. Many other examples of predictable shark behavior

could be cited but these suffice to belie the statement that "above all, the shark is unpredictable."

SHARKS AS EXPERIMENTAL ANIMALS

Sharks have long been used as laboratory animals (Gilbert 1969), and the great majority of physicians in the United States were introduced to vertebrate anatomy by dissecting a spiny dogfish, *Squalus acanthias*. This same shark has also been used extensively as an experimental animal in biomedical and physiological research for it is relatively small, can be worked on without being anesthetized, and is readily available at seaside laboratories at certain seasons of the year.

But is a shark really healthy after capture on hook and line and confinement in a pen or live car without feeding for days or even weeks? This question prompted one of my graduate students, Fred Martini at Cornell University, to study in same detail the metabolic changes that occur in *Squalus acanthias* as a result of capture and confinement. The results of his study, presented in another section of this volume, must give pause to those who use the species as an experimental animal, assuming they are working with a healthy shark. This raises a challenging question. How does one capture and maintain a shark in a healthy state for use as an experimental animal? Unfortunately, adequate experimental facilities for the study of sharks are few in number and expensive to maintain. Two of the finest are located at the Lerner Marine Laboratory (LML), Bimini, Bahamas (Figs. 3, 4), and the Mote Marine Laboratory (MML), Sarasota, Fla. (Figs. 5, 6). The LML is closed and the MML may soon have to abandon its experimental shark pools unless more funding for the facility is forthcoming. Such facilities at LML and MML have enabled investigators to study the behavior and response patterns of sharks under more controlled conditions than are possible in the open sea. This research complements that made in the natural environment. Both approaches are essential to an adequate understanding of shark behavior.

Open-sea studies are at best difficult, and relatively few scientists have left the confines of their laboratories to study sharks in their natural environment. Greater emphasis, however, should be placed on this approach because behavioral studies of sharks in the open sea promise rich rewards to trained observers who venture from their laboratory benches (Zahuranec 1975). The role sharks play in the ecology of coral reefs, or the behavior of certain sharks that appear to stake out territories and defend them from intruders are challenging subjects for investigation.

SHARKS AS A HAZARD

Patently, people are not the favorite food of sharks, for the number of attacks per year in the entire world is certainly less than 100, no more than 30% of which are fatal (Gilbert 1968b, Baldridge 1974). This figure

Figure 3   Lerner Marine Laboratory, Bimini, Bahamas, in 1960.
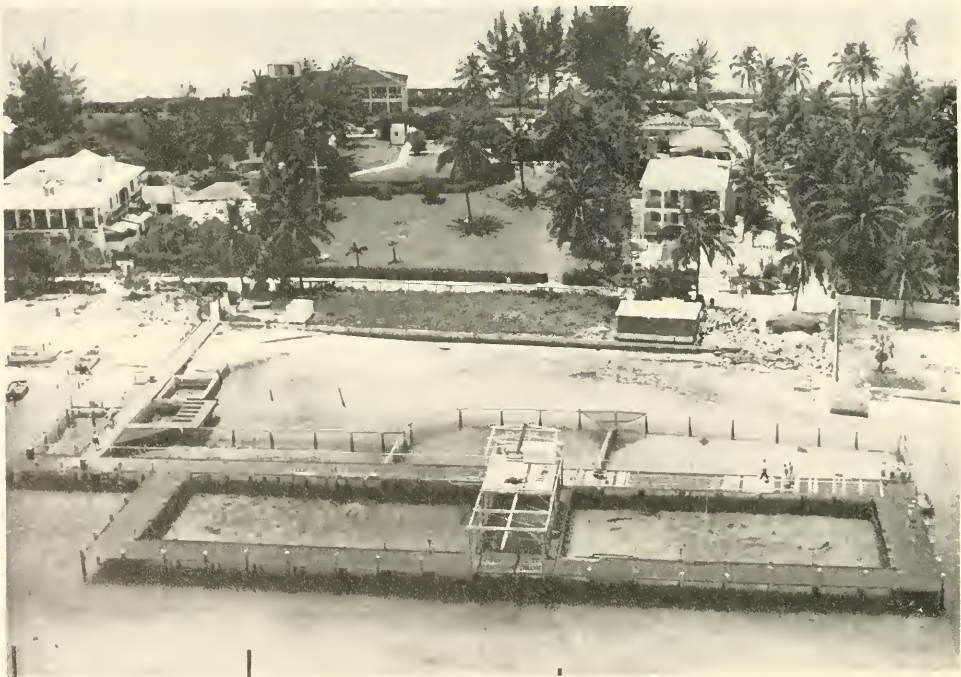
Figure 4   The shark pens at the Lerner Marine Laboratory. Two 12.2 m × 24.4 m
(40 ft × 80 ft) observation pens are separated by a gated 12.2 m × 6.1 m (40 ft × 20 ft)
operating pen. Additional fish pens are located along the upper (west) side of the shark
pens. (Photograph by Dade W. Thornton.)

Figure 5 Air view of the experimental shark facility at the Mote Marine Laboratory, Sarasota, Fla.

pales into insignificance when compared with other improbable accidents such as death from lightning or from the sting of a wasp or a bee. But shark attacks are gruesome, and the press frequently publicizes each gory detail.

While the news media unquestionably are culpable, public officials and representatives of chambers of commerce who insist on ignoring and concealing shark attacks are equally at fault. Until public officials in the United States recognize the problem and act on it, as in Australia and South Africa, our beaches will continue unpatrolled, our lifeguards will be untrained in first aid for shark victims, and visitors will not even have signs to alert them to the possible hazard. Bathers would feel far safer and more reassured if they knew their beaches were patrolled and that sensible precautionary measures were being taken by city officials to reduce the shark hazard. Although sometimes costly and imperfect, many methods to reduce the shark hazard exist (Springer and Gilbert 1963). It would appear that chemical deterrents are impractical simply because such vast quantities of material must be used to compensate for dilution (Baldridge 1976). Physical devices hold greater promise as shark deterrents and a number of such devices are now available (Gilbert and Gilbert

Figure 6 The experimental shark facility at the Mote Marine Laboratory consists of a circular channel (foreground) 24.4 m (80 ft) in diameter, connected by a 3.0 m × 6.1 m (10 ft × 20 ft) gated flume to circular pool 15.2 m (50 ft) in diameter. An electric hoist and observation platform above the flume permit efficient handling and observation of sharks up to 4.6 m (15 ft) in length.

1973, Gilbert 1976). Most important, however, is the need for adequate financial resources for research that will provide us not only with a better understanding of the biology and behavior of sharks but also with a knowledge of the many constructive uses to which man may put them (Gilbert 1968b).

THE PERSPECTIVE

A symposium, organized by Dr. Glenn Northcutt, was held in New Orleans June 3-4, 1976, on recent advances in the biology of sharks. It was the consensus of participants that the shark is a highly successful vertebrate— a splendid creature—admirably adapted to its environment and one from which we may learn much about our own rich structural and functional heritage. Let us therefore place the shark in perspective, for few are dangerous and, in the process of learning to cope with them, we will find that sharks contribute to a better understanding of humans and of some of the ailments that plague them.

ACKNOWLEDGMENTS

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II VISION

VISUAL SYSTEM OF THE ELASMOBRANCHS:
STATE OF THE ART 1960-1975

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INTRODUCTORY REMARKS

Systematic study of the shark's visual system dates back at least 200 years. Then, as now, sharks were considered interesting, even valuable, and thus worthy of study. Already in 1818 Soemmering had described the tapetum lucidum of sharks; the autonomic, direct action of light on the iris was demonstrated in 1839 (Rochon-Duvigneaud 1943). Until the middle of the 20th century the major research effort was toward understanding the gross and microscopic anatomy of the visual system. For example, no fewer than eight studies on elasmobranch retinal histology were published between 1890 and 1905 (Duke-Elder 1958).

The field of elasmobranch vision through the 1930's was dominated by the views of M. L. Verrier in France and V. Franz in Germany. Both had studied the visual systems of elasmobranchs for years, and both made important contributions (Verrier 1930, Franz 1934). Since then, several reviews (Walls 1942, Rochon-Duvigneaud 1943, 1958, Duke-Elder 1958, and Gilbert 1963) have summarized knowledge of the elasmobranch visual system. The usual approach was to break the eye into component parts such as retina, lens, cornea, etc., and discuss each, concentrating mainly on structural aspects (due no doubt to the paucity of physiological data). Thus these articles present an overview of the visual system in sharks as it was known 15 years ago.

It is not our intention to rehash this information and present again a "dissected view" of the elasmobranch eye. Rather, we shall review and synthesize research on the elasmobranch visual system published since the last review (Gilbert 1963). The scope of this article is further limited by the other contributions to vision in this volume. Thus, we have avoided reviewing the areas of refractive error, optics, accommodation, and higher visual centers in the central nervous system (CNS). This review is therefore limited to investigations published in the past 15 years on anatomy, biochemistry, physiology (including psychophysics) and natural history of photoreception in the elasmobranchs.

LATERAL EYES

Ocular Adnexa

Eyelids—Little modern work has been done on the ocular adnexa of elasmobranchs. Yet the palpebral complex (upper and lower lids, nictitating membrane) immediately separates elasmobranchs from bony fish. While a lacrimal system is unknown, elasmobranchs have well-formed eyelids that are mobile in some species, i.e., *Ginglymostoma* and *Cephaloscyllium*, but relatively immobile in others. In still other species the lower lid is secondarily folded longitudinally into a third eyelid (Figure 1), the original lower lid forming a structure similar to the nictitating membranes of amphibia, birds, and mammals. However, unlike the transparent nictitating membranes of terrestrial vertebrates, that of sharks is dense and opaque and its outer

Figure 1   *Negaprion brevirostris* with its nictitating membrane partially retracted. The nictitating membrane is most highly developed in *Negaprion* and the other carcharhinid sharks. (Photo by E. Fisher.)

surface is covered with dermal denticles. Gilbert (1963) reported that 31% of a representative 42 shark genera possess mobile third eyelids. Actually, nictitating membranes are known from only four shark families. They are especially well developed in the Carcharhinidae (Gilbert 1963).

Gilbert and Oren (1964) discussed the relation of the nictitating membrane to the so-called subocular fold found, for example, in the Triakidae. Based on the insertion of a muscle homologous to *levator palpebrae nictitans*, they contended that the subocular fold is a poorly developed nictitating membrane. They thus recommended dropping the term "subocular fold."

Movement of eyelids, including the nictitating membrane, has been described by Bell and Satchell (1963). Using mainly *Squalus*, but also with *Cephaloscyllium* and *Galeorhinus*, they stimulated the skin around the eye by various means. Such mechanical or electrical stimulation elicited an immediate unilateral and reflexive movement of the eyelids. Closure was slight in *Squalus* but complete in the other species. Bell and Satchell thus mapped the reflexogenic zone and measured such response properties as latency facilitation and rate of response. They presented excellent photographs of the response. In addition, they recorded from cranial nerves with micropipets and attempted to follow the neural pathway of the response.

Bell and Satchell concluded that the response may be interpreted as protective of the cornea. Clearly it is not visually mediated since it persists in a preparation with eyes removed. Neither is it part of a labyrinthine reflex. Gilbert (1963) and Harris (1965) also believed that eyelid closure was a protective mechanism.

Walls (1942) mentioned that blue sharks (*Prionace*) were seen to blink in bright light, but we have not been able to confirm this either in personal observations on the blue shark or by intensely illuminating the eyes of a lemon shark. On the other hand, movements of the nictitating membrane during feeding have been observed repeatedly in many species. Thus it seems clear that eye closure in sharks functions neither to lubricate the cornea which is bathed in an aquatic medium nor to reduce the amount of light entering the eye.

Agalides (1969) attempted to measure the sensitivity of the lemon shark (*Negaprion*) to electric stimuli by using a reflexive movement of the nictitating membrane similar to that described previously. Few details were given; however, Agalides estimated that the nictitating membrane will move if each ampulla of Lorenzini receives an electric stimulus of about 2×10^{-4} A.

Gruber and Schneiderman (1975), using the same response, i.e., unconditioned movement of the nictitating membrane to externally applied electric stimuli, studied acquisition, extinction, and other parameters of classical conditioning in the lemon shark (*Negaprion*). This response had been used before in psychophysical studies by Gruber (1966, 1967, 1969), and details on reliability of training as well as the precise form of conditioning were required to enhance the validity of the visual data. The basic conditioning trial consisted of a 500-ms flash of white light, the final 100 ms accompanied by an electric shock. The interval between trials averaged 30 s and 100 trials were given per session. All trials were videotaped and replayed in stop-frame mode for temporal analysis.

The experiment demonstrated that *Negaprion* could be reliably and quickly conditioned to move its nictitating membrane in response to a flash

of light by repeatedly giving such trials. The conditioned nictitating membrane response was shown to be a reliable indicator of both learning and detection of visual stimuli by the sharks. This study also demonstrated how rapid the movement of the nictitating membrane is: latency between onset of electrical stimulus and unconditioned movement of the nictitating membrane was less than 34 ms. In a well-trained shark, the conditioned response occurred only 190 ms after onset of light.

Extraocular Muscles/Eye Movements—Oliva (1967) reported on the topography of eye muscles in eight elasmobranch species. This ecological and phylogenetic study attemped to show a relation between habitat, extrinsic eye muscle, and external form of the eye. Oliva found that littoral sharks and Rajaformes have smaller eyes and better developed extrinsic eye muscles than pelagic elasmobranchs. The forms of eye muscles in various elasmobranch species, including origin and insertion, are clearly diagrammed; this appears to be the main value of this paper.

Bell and Satchell's (1963) study reported that stimulation of the snout of *Squalus* causes a reflexive rolling of the eye backwards and inwards. The function of this abduction, produced solely by contraction of the external rectus, was analogous to eye closure in *Mustelus* and *Cephaloscyllium*. That is, it serves to protect the shark's cornea.

Harris (1965) studied the eye movements of *Squalus* in detail. Eye movements of free-swimming sharks were recorded by cinematography after small plexiglass rods were glued onto each cornea. The rods served to amplify angular movements of the eye and acted as reference points. Studies made on restrained animals involved measurement of visual fields including blind areas and the relation of eye movements to passive body bending.

Five categories of eye movements were thus identified: (1) compensatory eye movements, i.e., those caused by static labyrinthine influences; (2) swimming movements, an active process opposing compensatory movements; (3) turning eye movements, which predicted a change in swimming direction; (4) fine movements possibly similar to slow saccades; and (5) the protective eye reflex just described. Other categories of eye movements were seen under artificial restraint. One important finding was that no visual stimuli had any immediate effect on eye position and no visual fixation of any type was ever observed. This agrees with our casual observations on *Negaprion*.

Harris showed that the visual field of an active shark contains a large component of binocular overlap (45°), but a blind spot is created by the bulge at the pectoral girdle which is enhanced by the 20° lateral misalignment of the eyes. This blind spot, amounting to 60° of visual angle below the fish, is completely eliminated by normal head and body movements associated with swimming. Thus for each complete stroke cycle the shark has nearly panoramic vision. This description casts some doubt on Hobson's (1964) functional interpretation of exaggerated swimming modes in gray sharks (Carcharhinidae). Hobson suggested that the highly serpentine movements might aid in increasing the visual field. However, if Harris's calculations are correct, exaggerated head movements are not necessary; indeed,

they tend to destroy stabilization of the visual field produced by normal eye and head movements.

The Whole Eye—The eyes of most elasmobranchs are prominent and are placed laterally on the head and set for some degree of binocular overlap. One exception may be the hammerheads (Sphyrnidae) whose visual fields apparently do not overlap (Walls 1942). The size of the globe varies from less than 1% of total length in the Orectolobidae to several percent in the deep sea squaloids. Sharks with completely degenerate eyes are unknown.

Figure 2, the eye of *Negaprion* sectioned in horizontal and vertical plane, demonstrates that the globe of sharks is not spherical; it is strongly ellipsoidal, being most compressed in the anteroposterior axis.

Details of the whole eye relative to image formation and optical landmarks are covered elsewhere in this volume.

The Cornea

Light enters the eye through the cornea, a more structurally organized continuation of the fibrous outer layer, the sclera. In addition to its optical properties, the cornea must withstand intraocular pressure from within and protect the eye from without. It is distinguished from other ocular tissues by its anatomical position. The cornea is the interface between eye and environment, with all the concurrent problems of water and ion flow, but, since it must remain transparent, blood vessels are absent. This poses a distinct problem for nutrition (Maurice and Riley 1970).

In several vertebrate classes the cornea is modified, for example, as an ocular filter. In contrast, the cornea of elasmobranchs is structurally and optically simple. While it possesses all the usual vertebrate layers, including Descemet's membrane (not usually found in teleosts), one attribute renders this cornea unique among vertebrates: it does not swell in distilled water. This is unexpected, not only because most other corneas swell but also because of the high osmotic pressure in elasmobranch tissue. This simple property, long ago recognized by Ranvier (1878), means the elasmobranch cornea remains transparent under a variety of conditions. Clinically, the properties of low water uptake and resistance to opacity make the elasmobranch cornea ideal for use in heterograft transplants. Payrau (1965, 1969) reported that this cornea is well tolerated by hosts, and several shark-human transplants have been made. Actually, the elasmobranch cornea differs from other vertebrate corneas in many interesting anatomical, biochemical, and physiological features (Obenberger et al. 1971a).

Anatomy—Smelser's (1962) redescription of the nonswelling properties of the elasmobranch cornea created renewed interest in the structure of this tissue. Faure (1970), reporting on the embryonic development of the cornea in *Scyliorhinus*, made detailed observation at the optical and electron microscope level on three growth stages: the 30-mm and 75-mm embryo and the 140-mm (5-m) "young dogfish." In the 30-mm stage the completely ectodermal cornea is an acellular secretion of the epithelium. Early on, the
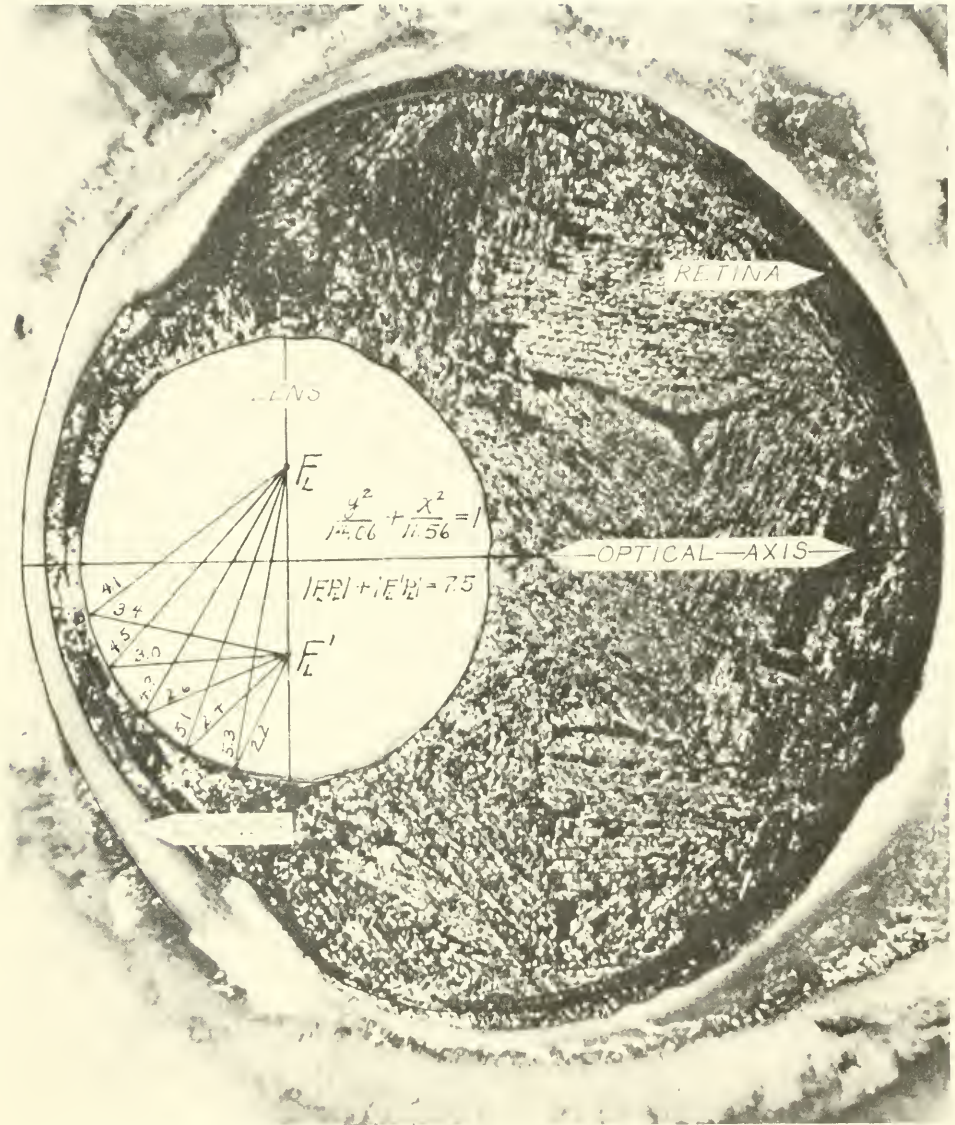
Figure 2 Frozen section of the eye of *Negaprion brevirostris* cut in the equatorial plane. The asymmetry of the eye and lens is clearly shown. The shape of the lens can best be described as an ellipse as the calculations indicate. (Photo taken from Hueter and Gruber, unpublished data.)

stromal "anlage" becomes organized roughly as in the adult. This anlage serves as a matrix for deposition of collagen fibers and directs migration of fibroblasts during ontogenesis. The development of all layers of the adult cornea, except corneal endothelium, was described.

In a detailed anatomical treatment, Goldman and Benedek (1967) described the organization of the cornea in *Squalus* and agreed in principle with Ranvier's observation that the nonswelling properties could be completely understood by the anatomical arrangement of "sutural fibers" running from Bowman's membrane to the posterior surface of the cornea. Goldman and Benedek stated that of all vertebrates possessing corneas with lamellated stromata the elasmobranchs have the most primitive. The elasmobranch cornea is, however, clearly advanced over the condition found in the cyclostomes.

Six specific layers can be recognized in the cornea of *Squalus:* the epithelium, in contact with sea water; the basal lamina; Bowman's layer; the *substantia propria* or stroma; Descemet's membrane; and a monolayer of mesothelial cells in contact with the aqueous humor (Figure 3). The cornea of man is similarly layered. Tolpin et al. (1969) give the corneal thickness in *Squalus* as 0.25 mm, increasing by about 30% toward the periphery. Harding
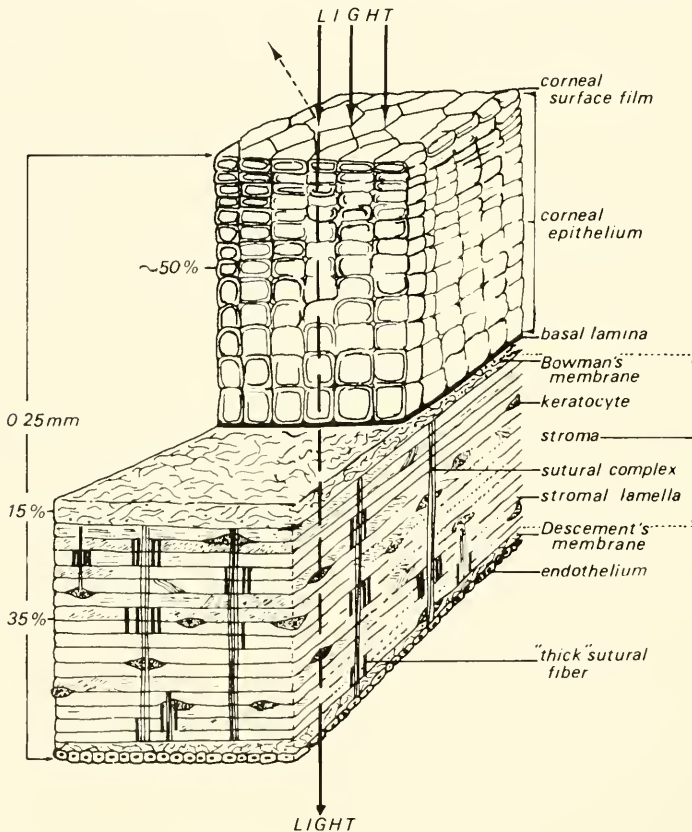
Figure 3   Main features of the elasmobranch cornea. The layering is typical but the proportions (%) differ from those in other vertebrates. The most noteworthy feature is the sutural complex that maintains corneal transparency under harsh conditions. The diagram, derived from several sources, is a composite of the corneas of *Scyliorhinus* and *Squalus.*

et al. (1974) have described the external surface of the cornea in *Mustelus* and *Raja*, finding structures very similar to those of mammals but quite different from those of teleosts. The surface is covered with 0.2-μm wide microprojections which probably represent microvilli. In mammals, analogous microprojections are thought to aid in holding the tear film in place, but Harding et al. questioned this function for sharks. They noted, however, that if ocular secretions were viscous enough, these projections could provide some mechanical support and thus a stable optical surface. Certainly, the microprojections increase surface area, thereby aiding in diffusion processes probably necessary for aquatic corneas.

The epithelium comprises about 50% (0.101 mm) of the corneal thickness. In comparison, the human corneal epithelium is only 10% of the corneal thickness (Maurice 1969). The basal layer of epithelial cells is separated from Bowman's layer by a thin basal lamina similar to that of man. It is in this layer that the sutural fibers are anchored. Zigman et al. (1965) indicate that the corneal epithelium of the dogfish (*Mustelus*) contains approximately 12 layers of DNA-rich cells.

Bowman's layer is comprised of a randomly oriented collagen fiber feltwork, again similar to that of humans. The fourth layer, known as the stroma or *substantia propria*, is about 0.07 mm thick and contains 25 lamellae. The lamellae are composed of extremely regular, dense collagen fibers embedded in a gelatinous matrix similar to aqueous humor. The lamellar ribbons are more nearly parallel than those of mammals and do not interweave. This regularity would appear to be the most important optical property of the cornea.

The transparency of the cornea has been explained by Maurice (1957) on the basis of an "interference theory." Maurice rejects the idea that the cornea has a uniform index of refraction. Rather, the stromal fibers are arranged in a regular semicrystal lattice with spacing of less than a single wavelength of light and thus behave as a diffraction grating. The overall effect is to suppress, by destructive interference, diffuse scattering of light and to favor forward transmission. The main problem with this hypothesis is that the relatively thick, randomly arranged fibers of Bowman's layer in *Squalus* were shown by slit lamp examination (Goldman and Benedek 1967) to scatter less light than the stroma. Thus, a regular lattice structure is not a necessary condition for corneal transparency. In response to this criticism, Maurice (1969) speculated that the degree of disorder tolerated by an interference mechanism might be considerable if the fibril axes were nearly equidistant.

In man the cornea is the principal refracting element of the eye and accounts for 75% of normal refraction (Maurice 1969). However, in aquatic animals the refractive role of the cornea is severely reduced because seawater and cornea have very similar indices of refraction. Consequently, most of the refraction takes place in the large ocular lens.

Goldman and Benedek (1967) describe the cellular elements of the dogfish stroma—keratocytes—as "quite similar to keratocytes found in normal human and rabbit corneas." (p. 591). Keratocytes, which are modified

fibroblasts, make up a small fraction of the stroma and are usually located between lamellae. Where they occur they maintain contact through cellular processes, forming a virtual line of cells. Keratocytes appear to migrate through the cornea, and their vertical processes, i.e., those on the optical axis, are often in close association with the unique sutural fibers.

The fifth layer is Descemet's membrane, a homogeneous structure of loosely interwoven fine fibrils only 400 nm thick. In man, this structure is much thicker and probably represents the hypertrophied basement membrane of the underlying cellular layer (Maurice 1969). The most posterior (vitread) layer is called the endothelium. However, the term "endothelium" ordinarily refers to tissue lining the heart, blood vessels, and lymphatic system, while the term "mesothelium" is reserved for mesenchymal epithelium lining body cavities. For this and other reasons, Walls (1942) felt that endothelium was an inaccurate term for "corneal mesothelium." Donn (1966) reviews the arguments for renaming this structure, but because "endothelium" is so widely accepted Maurice (1966) sees no purpose in changing the name.

Existence of a corneal endothelium in lower vertebrates has long been denied, but Gilbert (1963) correctly insisted that sharks possess this corneal layer. Goldman and Benedek observed the endothelium in flat histological sections and by examination of the cornea of live sharks with a slit lamp. Although an endothelium lines the cornea of *Squalus*, it is only one cell layer thick and very easily lost in histological preparation; this probably led to the disagreement. In higher vertebrates, endothelial mechanisms regulate hydration of the stroma. Thus, destruction of the endothelium results in deleterious corneal edema. In elasmobranchs, however, the structural arrangement provided by the sutural complex makes any hypothetical endothelial "pump" unnecessary.

It is the sutural fibers that set the elasmobranch cornea apart from those of most other vertebrates (except see Van Horn et al. 1969a, 1969b). The sutural complex of *Squalus* and *Scyliorhinus* contains two types of fibers: a principal bundle of fine fibrils and an accessory group of thick fibers. The fine fibrils run a straight course through the entire cornea; the thick fibers span at most 2–3 lamellae. There are about 15 sutural complexes per square millimeter. Thin fibers originate in the basal lamina, extend down through Bowman's layer and are joined at the stroma by thick fibers. After coursing through the lamellated stroma the fibers terminate in Descemet's membrane. The fine fibrils are histochemically similar to reticulum, but their exact nature is unknown. The thick fibers are thought to be typical collagen (Obenberger et al. 1971a). While biochemical mechanisms are perhaps involved (Moczar et al. 1969), the sutural fibers could completely account for the nonswelling properties of the cornea. When the corneas of other animals swell, the collagen fibers in lamellae separate, as do the lamellae themselves. The sutural complex appears to mechanically restrain stromal elements from separating, thus maintaining the intrinsic structure.

Physiology—In elasmobranchs there is a need to prevent loss of water, rather than guard against its entrance as in many other vertebrates.

Smelser (1962) showed that the naked stroma of *Mustelus* loses up to 15% of its weight (mostly water) when immersed in distilled water for 1 h. The stroma of the scup, *Stenotomus*, a marine teleost, swells by 350% under the same conditions. Obenberger et al. (1971*a*) investigated details of corneal hydration in *Scyliorhinus*. They confirmed that the elasmobranch cornea actually loses weight when immersed in a variety of liquids. They also observed marked solubilization of solid components, the cornea dissolving up to 22% in distilled water. In a second study, Obenberger et al. (1971*b*) found that the cornea could be made to swell in media of low pH. Paradoxically, a pH of 4 is the point of minimal swelling in mammals.

Tolpin et al. (1969) investigated the relation between swelling pressure (force per unit area needed to maintain constant corneal thickness) and hydration of the cornea in *Squalus*. The normal value of corneal hydration (3.2 mg H_2O/mg dry wt of the cornea) does not differ from that of mammals. The main difference is that normal swelling pressure for *Squalus* is 0.0 mm Hg while the normal value for mammals is 50 mm Hg. This means that at maximal swelling, forces in the dogfish cornea are exactly balanced (presumably) by the sutural fibers. In contrast, the mammalian cornea imbibes up to 12 times the normal value of water before swelling ceases. These results confirm that a corneal fluid transport mechanism, important in mammals, need not operate in sharks.

Physiological investigations by Edelhauser (1968) provided data on the passage of water and salts through the cornea. According to Edelhauser, the aquatic cornea is devoid of a tear film and thus the problem of water and ion passage across this tissue can be critical (but see Harding et al. 1974). The problem of water and ion flow is intimately bound up with the adaptation of elasmobranchs to life in a salty medium. It is well known that elasmobranchs have achieved "osmotic superiority" in the marine environment by storing huge amounts of urea and trimethylamine (oxide) in their body. Their environmental situation is thus somewhat analogous to that of freshwater fish: the external medium in which sharks live is relatively hypotonic to their internal medium.

Edelhauser found the cornea resistant to water and sodium influx from the environment. No net movement of radioactive sodium (Na^{23}) or tritiated water (H_2^3O) across the cornea was observed, regardless of osmotic gradient. The thick epithelial layer of the cornea appeared to offer the greatest resistance to passage of materials. Impermeability to sodium and water is typical of the aquatic cornea; the aerial cornea is permeable to these materials. Harding et al. (1974) reported the existence of a viscous precorneal film, presumably in elasmobranchs and certainly in teleosts. Some of the resistance to transport across the cornea could reside in this coating.

Biochemistry—The chemical composition and biochemical reactions in the cornea do not differ significantly from those of other connective tissue. However, the mucopolysaccharide content does form a cornea-specific pattern (Maurice and Riley 1970). The importance of corneal glycoproteins

relates to water imbibition and swelling properties. For example, Maurice and Riley (1970) attribute corneal swelling directly to repulsion of negative charges found on mucopolysaccharide molecules. Anseth (1961) suggested that the glucosamine:galactosamine ratio might be critical to the question of water uptake, since it is high in animals that exhibit limited corneal swelling. Because of the importance of mucopolysaccharides in water uptake and because of the unusual swelling properties of elasmobranchs, several authors (Suzuki 1960, Mathews and Inouye 1961, Robert and Schillinger 1967, Moczar et al. 1969, and Praus and Goldman 1970) have investigated these sugar-protein complexes in the cornea. Robert and Schillinger (1967) demonstrated that elasmobranch and teleost corneas were biochemically dissimilar and that the cornea of *Scyliorhinus* contained much more insoluble protein than those of teleosts. They suggested that the high content of keratoglycosaminoglycan in the shark played a role in maintaining transparency. Moczar et al. (1969) came to a similar conclusion in a more detailed study on cod, whiting, and dogfish (*Scyliorhinus*). They felt that resistance to swelling might be related to insoluble mannose-containing glycoproteins in the stroma. The cornea of the *Scyliorhinus* had the highest percent dry weight, lowest hydration, most insoluble stroma, and lowest mucopolysaccharide-protein observed in their study. Praus and Goldman (1970) found a significant difference between corneal mucopolysaccharides of mammals and *Squalus:* galactosaminoglycan (chondroitin sulfate) predominates by 75% over glucosaminoglycan (keratin sulfate) in the shark. Thus, the shark cornea more closely resembles shark cartilage, which contains more than 90% chondroitin sulfate. Exactly how chondroitin sulfate is associated with nonswelling properties of the elasmobranch cornea is the subject of future studies by Praus and Goldman.

The Choroid

The choroid coat may be considered that part of the uveal tract which lies just internal to and lines the sclera. In most vertebrates it consists of connective, vascular, and pigmented tissue. In elasmobranchs the uvea is the only vascularized tissue in the adult eye. According to Walls (1942) two blood vessels enter the globe: a temporal choroidal artery and a ventral artery supplying the iris. The eye is drained by two main veins, one dorsal and one ventral. François and Neetens (1974) briefly summarized the vascular supply of the elasmobranch eye.

In some species the choroid is thickened by a tangle of blood vessels, connective tissue, and possibly lymphatic spaces known collectively as the *suprachoroidea.* The entire outer part of the uvea has been called the *epichoroid* (Duke-Elder 1958). The inner part, lining the retinal epithelium and supplying the retina with nutrition, is the *choriocapillaris.* Between lies the unique choroidal tapetum of the elasmobranchs.

Tapetum—The tapetum lucidum is a specialized ocular structure responsible for the eyeshine of animals. Eyeshine is widely distributed among

the arthropods and vertebrates, including elasmobranchs, whose tapetum consists of a series of reflecting plates in the choroidal layer behind the retina. These plates and associated melanocytes, known collectively as the *tapetum lucidum choroidale* (Figure 4), have been well studied over the last 10 years. Gilbert (1963) presented a rather complete review of the elasmobranch tapetum and suggested a need for further inquiry into mechanisms of tapetal occlusion. In a series of independent investigations, both Nicol and Kuchnow addressed themselves to some of the questions Gilbert raised.

One of the most unusual features of the elasmobranch's tapetum is its ability to darken. This so-called occlusability, described by Franz (1931), was never subjected to experimental verification. During occlusion, light-screening pigments are said to migrate over the reflecting plates, darkening the tapetum.

Anatomy and Function—To confirm this proposed mechanism Nicol (1961) investigated structure and occlusability of the tapetum of *Scyliorhinus*. The eyes of experimental animals held under various light regimes were removed for gross observation and histological study. Experimental results disagreed with Franz' (1931) original proposal. Nicol's data clearly demonstrated that the tapetum of *Scyliorhinus* does not darken, i.e., is not occlusable. Histochemical tests on tapetal pigments suggested that the light-screening material was melanin; the reflecting pigments were thought to be guanine, but chemical tests were inconclusive. One final observation in the 1961 paper was that photoreceptors underlying the permanently bright tapetal stripe were twice as long as elsewhere in the retina.

The failure of Franz' occlusion theory for *Scyliorhinus* prompted Nicol to continue this work, and he eventually published observations on some 20 elasmobranch species. Quantitative results on percent reflection and kinetics of occlusion in several species, most notably *Squalus acanthias*, were

Figure 4 The *tapetum lucidum choroidale* in *Squalus acanthias*. Environmental light entering the eye and passing through the retina continues through the retinal epithelium (RE), which unlike that of most vertebrates, is devoid of screening pigment. Light then passes through the nutritive *choriocapillaris* (CC) to impinge on the tapetal plates (TP). If, as in darkness, the melanin pigment (M) is withdrawn into the pigment cells (PC), light will reflect from the reflecting crystals (RC) back through the retina. This arrangement produces eyeshine in sharks. (Modified from Best and Nicol (1967). (Reproduced by kind permission from the authors and the editor of *Contributions in Marine Science*.)
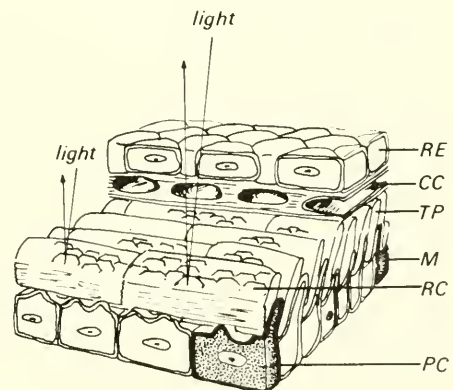
reported (Nicol 1964). A summary of results led to provisional grouping of elasmobranchs on the basis of tapetal occlusability. Nicol thus recognized

1) The occlusable tapetum, which darkens when pigments migrate over the reflecting plates, found in pelagic species from well-illuminated waters;

2) The nonocclusable, permanently bright tapetum occupying the entire fundus, found primarily in deep-sea squaloids;

3) The nonocclusable tapetum occupying part of the fundus, occurring in certain littoral and benthic elasmobranchs (the condition of *Scyliorhinus* is typical. Its tapetum is wanting ventrally, but where it exists it is permanently bright);

4) The partly occlusable tapetum, also found in benthic elasmobranchs, characterized by partial expansion of the tapetal melanophores in the light-adapted eye.

Thus, even concerning the apparently simple question of tapetal occlus-ability, the existing situation turned out to be rather complex.

Detailed anatomical, physiological, and physical properties of the tapetum were reported by Denton and Nicol (1964). Their histological study pro-duced two noteworthy observations: (1) tapetal plates are aligned differently in different parts of the eye so as to reflect light directly back through the same optical path of entrance (Figure 5) and (2) nonpigmented processes of the melanophore cells appear to be permanently extended in fixed pathways over the tapetal plates. Denton and Nicol thus suggested that pigment gran-ules, not the entire pigment cell, migrate during occlusion. This agrees with the earlier ideas of Bernstein (1961) and Gilbert (1963) and later with Kuchnow and Martin (1970*a*).

Denton and Nicol also studied kinetics of occlusion in *Squalus*, using fixed and fresh tapeta. The occluded (light-adapted) tapetum becomes completely shiny after 1 h in darkness; the tapetum of a dark-adapted dogfish darkens after about 2 h in bright light. Pharmaceutical agents and anesthetics had little or no effect on expansion of pigments in isolated tapeta. Stripping the

Figure 5   Section through the center of the eye of *Prionace glauca* showing the orientation of tapetal plates behind the retina. The guanine-coated plates are so arranged as to reflect light back through the path of entry. (Modified from Denton and Nicol (1964). By kind permission of the authors and the *Journal of the Marine Biological Association of the United Kingdom* © 1964 Cambridge University Press Limited.)
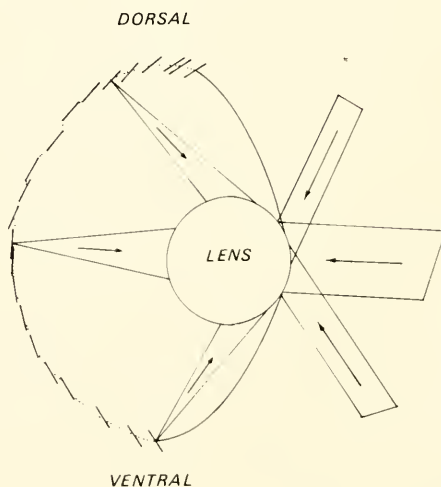
retina from the tapetum also caused melanophores to expand at the same rate and to the same extent as in an eye placed in bright light. The pigment of such a preparation does not retreat again when returned to darkness. Though it is not specifically mentioned by Denton and Nicol, this condition signals neuronal or hormonal communication between retina and choroid.

Perhaps the most noteworthy feature of the elasmobranch tapetum is that its reflection is specular, i.e., more like a mirror than a diffusing screen. Reflection approaches 90% at certain wavelengths, a value far exceeding that of cats, for example (Weale 1953). In addition, reflection from the tapetum is spectral, and eyeshine in different species of sharks varies from blue green to golden.

As stated, histological observations demonstrated that throughout the eye the tapetal plates are roughly perpendicular to the light that can reach them. This has the important consequence of reflecting light back through the lens into the environment, which optically reduces glare from tapetally scattered light within the eye while maintaining any visual advantages the tapetum might confer. Denton and Nicol (1965) confirmed this anatomical finding by directly measuring the orientation of the tapetal reflecting surfaces in excised and opened eyes. Their measurements were in complete accord with the earlier histological results. The 1965 study was completed with a thought-provoking discussion of the advantages of tapeta. They first suggest a relation between habitat, primarily depth, and pupil mobility. To this is added tapetal occlusability. For example, *Scyliorhinus* and other nocturnal, bottom-dwelling, littoral sharks appear to have a highly mobile pupil, a permanently bright tapetum, and a black field in the ventral fundus. Denton and Nicol contrast them with the active, diurnal, pelagic sharks such as *Prionace* and *Squalus*. These animals have an occlusable tapetum but little iris movement. This point is questionable, however, since, for example, *Carcharhinus falciformis* and *C. longimanus* are pelagic, feed in daylight, and have highly mobile pupils. In addition, results from acoustical tagging (Nelson 1974) show that *Prionace* is very active at night, moving inshore presumably to feed and then moving offshore at dawn.

Denton and Nicol point out that the usual explanation for possession of a tapetum is that it imparts a twofold increase in sensitivity. But this cannot be the explanation for sharks. The authors determined concentration of visual pigments in the outer segments of various elasmobranch and teleost photoreceptors and found that the bony fishes (without tapeta) have about twice the optical density of photopigment in their rods. Thus, in a given environment the elasmobranch tapetum is not used for greater absorption of light (i.e., higher sensitivity) but rather to give about the same absorption compared to sympatric teleosts. This is because the shark photoreceptors contain only one-half the concentration of visual pigment. Denton and Nicol suggest two possible advantages of low pigment density combined with a tapetum:

1) The signal-to-noise ratio of the visual system would be improved with less photopigment since there would be less spontaneous bleaching and thus

fewer spurious signals (i.e., noise). At the same time, the tapetum would in effect increase sensitivity as if there were twice the amount of pigment,

2) The lower pigment concentration would permit more rapid dark adaptation while maintaining high sensitivity. Dark adaptation depends partly on regeneration of visual pigment and so the absolute amount of pigment could set the final limit on kinetics of dark adaption. However, Gruber (1967) and Hamasaki et al. (1967) have shown that the rate of dark adaption in several elasmobranchs is relatively slow.

Nicol (1965) turned his attention to physiological mechanisms subserving migration of the choroidal tapetal pigment. He stressed that pigment moves through fixed channels. The experimental work was designed to determine whether control of the tapetal pigments was achieved through direct action of light on an independent effector or whether neuronal or hormonal factors were involved. Methods included nerve sectioning, ablation, and pharmaceutical assays. Results of at least 14 separate experiments apparently precluded extraocular neuronal or hormonal control of the tapetal system. However, as we have already seen, a tapetum devoid of retina darkens permanently. Thus the pigment cells cannot be labeled as exclusively independent effectors. In addition, Nicol found that physically replacing the retina on a dark-adapting tapetum inhibited the expansion of pigment. Because of the anatomical separation between retinal receptors and tapetal pigment cells, Nicol rejected the notion of direct retinal control of pigment migration. Thus, although many possibilities were ruled out, the actual mechanism was not revealed.

Kuchnow (1969a, 1969b), already measuring kinetics of tapetal light and dark adaptation, realized that some type of interactive mechanism was indicated with retinal control over pigment aggregation. Kuchnow and Martin (1970a) thus looked at the fine structure of the melanocytes in *Mustelus* to determine whether neuronal elements were present. Previous workers (Bernstein 1961, Best and Nichol 1967) had failed to locate any nerve endings in the pigment cells. However, Kuchnow and Martin (1970a) were able to identify structures at the basal portion of the melanocytes that had the characteristics of nerve endings. Synapses between neurons and melanocytes with cleft distances of 30–40 nm were also identified. These findings provide strong evidence for neuronal control of pigment migration, but the mode of action is still an open question. The most difficult point to reconcile is how, by merely replacing the retina on a piece of "stripped" tapetum, pigment migration was halted.

Best and Nicol (1967) investigated ultrastructure, orientation, chemical composition, and reflection properties of the tapetal reflecting cells in *Squalus* and *Scyliorhinus*. The internal margins of the reflecting cells, i.e., the surface which reflects light back to the photoreceptors, appear in gross aspect to be irregularly overlapping rounded ellipsoids, somewhat like fish scales. The faces of these reflecting cells are 100×60 μm and crystals obtained from them are very thin, elongate hexagons. While light reflected from individual crystals varied in color, the overall effect of tapetal reflection

produced an unsaturated spectrum. For example, the tapetum of *Squalus* is bluish green, reflecting very efficiently (90%) in the middle wavelengths (500–515 nm) but falling off to 50% in the blue (420 nm) and red (650 nm). In a clear presentation, Best and Nicol explained the origin of individual crystal colors as well as the general coloration of the tapetum. As suspected, the high reflectivity in color is due to constructive interference in the multicrystal layer "thin-film" system of the tapetum. Thin-film systems are found elsewhere, such as in fish scales, wings of beetles, squid eyes, butterfly wings, etc. (Denton and Land 1967). Interference phenomena are also used extensively in optical instruments when relatively pure colors are required (i.e., interference filters, diffraction grating). Best and Nicol conclude with a detailed discussion on fine structure, orientation, and alignment of reflecting cells and crystals in the tapetum.

Biochemistry—Biochemistry of the tapetum was reported by Nicol and van Baalen (1968), extending the earlier work on composition of tapetal crystals. The authors eventually prepared a pure sample of crystal for analysis. An enzyme assay was used in which the tapetum was first treated with xanthine oxidase to convert residual xanthine to uric acid. Guanase was then added, converting guanine to xanthine, which goes over to uric acid from the previous enzyme addition. Increase in optical density at 290 nm indicated the presence and amount of guanine in the sample. The authors reported that choroid contains "astonishingly large amounts of guanine, nearly 1 mg/cm^2 in *Dasyatis*" (p. 76). Clean crystals from the tapetum of *Dasyatis* were secured by digesting the choroid in trypsin. The resultant enzyme analysis unequivocally demonstrated that these crystals were chemically identical to guanine. At least four fluorescent substances were isolated from the choroid but could not be absolutely identified. A fluorescent material though to be xanthopterin had previously been reported from the eye of *Squalus* (Pirie and Simpson 1946). The importance to vision of fluorescent materials in the tapetum has been discussed by Dartnall et al. (1965).

Another biochemically important material in the tapetum is the light-screening pigment responsible for occlusion of the light-adapted tapetal plates. While melanin has been associated with tapeta of other animals, Fox and Kuchnow (1965) point out that other dark pigments such as chromolipids could be substituted in the elasmobranch choroid and thus be confused with melanin. For positive chemical identification, they extracted the opaque choroidal pigment of *Prionace*, *Heterodontus*, and *Platyrhinoidis*. Melanin, however, is a rather complex and inert polymeric molecule and thus difficult to characterize. Their strategy was to try a number of different chemical tests (at least 14) to determine whether the extracted pigment had properties similar to those of melanin. They concluded that the screening pigment in all tests behaved quite like melanin and was thus chemically identical to that substance.

Kinetics of Occlusion—Kuchnow then turned his attention to the physiology of tapetal pigments (Kuchnow and Gilbert 1967, Kuchnow

1969*a*, 1969*b*). Most of these studies were done in vivo with the intact eye and thus differ from Nicol's approach. The method of measuring tapetal activity involved curarizing and fixing iridectomized animals in space, then photographing the eye under controlled illumination. Densitometry of the photographic emulsion compared with a standard gray scale was proportional to the amount of light reflected from the tapetum. While absolute values were unobtainable, Kuchnow believed that the photographic technique was simple and gave an accurate picture of the rate and extent of tapetal response. Laboratory experiments were performed on *Cephaloscyllium*, *Heterodontus*, *Mustelus*, *Negaprion*, and *Triakis*. Field observations were made on *Apristurus*, *Carcharhinus*, *Ginglymostoma*, and *Prionace*. As expected, the scyliorhinid shark *Apristurus* had a nonocclusable tapetum. The tapeta of all other species were occlusable to some degree. Laboratory experiments (mainly on *Heterodontus*) demonstrated that pigment begins to migrate in the dark-adapted shark when levels exceed 10^{-6} fL (3.4×10^{-6} cd/m^2) with graded responses up to 10^{-1} fL (3.4×10^{-1} cd/m^2). Footlamberts are photometric values and thus not the most appropriate measure of light for an experiment of this sort. Maximum pigment extension occurred at 10^{-1} fL. Conversely, tapetal plates remained occluded until the light fell below 1 fL. Maximum pigment aggregation occurred at levels just below 10^{-3} fL (3.4×10^{-3} cd/m^2). Data from a number of species indicated that light adaptation, i.e., response of the melanin to light, was graded, requiring 60–90 min for complete occlusion; dark adaptation was invariably faster, taking anywhere from 30 to 60 min. At onset of both light and darkness, a lag of several minutes was noted before change in reflectivity began. This lag represents movement of pigment granules through channels on the tapetal plates to an anatomical position were changes in light absorption can first take place.

Research on the elasmobranch tapetum has been especially fruitful. Of all animals, the elasmobranchs have the most elaborate tapetum (Pirie 1965). While other animals may have occlusable tapeta, none is known to combine sensitivity of occlusion, regularity of multilayer crystals, specific plate orientation, and specular reflection into such an ordered tapetum as do the elasmobranchs. Biochemistry of tapetal guanine and melanin are reasonably well known, but the actual mechanism(s) subserving tapetal occlusion remains to be discovered. Everyone seems to agree that tapetal pigment flows through fixed channels, and thus tapetal occlusion is not pseudopodal as had first been thought (Franz 1931). The primary unresolved question is: How is the tapetal pigment response triggered and controlled?

While we understand the basis of eyeshine in the elasmobranchs, speculation on its value to the organism has not been entirely convincing. Certainly, the tapetum is an important optical device, since eyeshine has evolved independently in many phyla and from a number of unrelated structures. The tapetum is clearly useful only in dim light. However, its value as a sensitivity mechanism alone must be seriously questioned on the basis of Nicol's (Denton and Nicol 1965) observation on the relation between density of visual pigment and possession of a reflecting tapetum. Simply stated, certain teleosts have twice the density of visual pigment compared to sharks

from the same habitat but lack a reflecting tapetum. All other factors aside, teleosts without tapeta and elasmobranchs with eyeshine have the same potential sensitivity. Nicol suggested that lower pigment density of sharks might permit more rapid dark adaptation, but measurement of this parameter (Gruber 1967, Hamasaki et al. 1967) has shown that this is a relatively slow process in elasmobranchs. The possibility of improving signal-to-noise ratio at the retinal level has been discussed. One final possibility not often mentioned involves camouflage. Light-absorbing pigments in the fundus confer the familiar dead black pupil characteristic of most vertebrate eyes. This "eye spot" has important behavioral consequences (Blest 1957). Some organisms have evolved conspicuous nonvisual eye spots, and many have evolved markings and patterns in an apparent effort to disguise their functional eye spot. Might it be possible that elasmobranchs to some extent use reflection from the fundus to hide their eye spot? Certainly the plate orientation tends to reflect light of all optical pathways back through the lens into the environment.

The Ciliary Zone

The ciliary zone, in the anterior segment of the eye, is an anatomically and physiologically heterogeneous zone of varied embryological origins. Bounded posteriorly by the *ora terminalis* (the termination of the sensory retina) and anteriorly by the corneal endothelium, the ciliary zone is almost everywhere bathed with aqueous humor. Structures making up the elasmobranch ciliary zone include the iris, the ciliary body, ciliary folds, and the ciliary papilla on which the lens rests. Zonular fibers and suspensory ligaments that hold the crystalline lens in place also occur but are thought to be condensations of the vitreous body (Duke-Elder 1958). The ciliary structures are composed of forward extensions of the nonsensory retina in intimate contact with the uveal (vascular) tract. Functions of the ciliary zone are many and varied. For example, ciliary structures control the amount of light entering the eye, probably move the lens in accommodation (see Sivak elsewhere in this volume), and secrete aqueous humor. These structures have lately received considerable attention because anatomical, physiological, and biochemical similarities between the ciliary apparatus of mammals and elasmobranchs suggest that the shark system may be the prototype for higher organisms (Doolittle et al. 1960, Maren 1962a, Jampol and Forrest 1972). In addition, elasmobranchs are among the few fish with the capacity for extensive pupillary movements.

Iris Anatomy—Kuchnow and Martin (1970b, 1972) investigated the fine structure of the iris in seven elasmobranch species. The older literature presented a confusing picture of the iris, especially regarding the association of neurons with contractile elements. Physiological experiments by Young (1933) demonstrated that the iris sphincter is responsive directly to light. This can easily be confirmed by excising a small piece of iris from a dark-adapted shark and exposing it to intense light. The in vitro piece of iris will vigorously contract. However, Young believed that the iris dilator was under

nervous control. Yet neurons contacting muscular elements had not been observed and only one investigator (Carrere 1922) actually described myofilaments from the irides of elasmobranchs.

Kuchnow and Martin (1970b) established that the elasmobranch iris possesses contractile elements with the characteristics of smooth muscle. These elements were composed of a single type of myofilament 4–10 nm in diameter. Neurons and neuromuscular junctions were also identified in both dilator and sphincter muscles. The finding of neuromuscular junctions in the sphincter was unexpected since it is an independent effector. The ratio of neurons to sphincter fibers was, surprisingly, very nearly 1:1.

The existence of neural elements suggested CNS control, and the authors cited evidence for a retinally mediated iridial reflex. In addition, they claimed that the iris dilator is cholinergically innervated by cranial nerve III. However, attempts to dilate the pupils of both *Negaprion* and *Gingly-mostoma* (Plate IIA) with adrenergic and cholinergic agents topically applied and even injected into the anterior chamber were unsuccessful (Gruber 1969).

Kuchnow and Martin (1972) next investigated the unique iris of skates (*Raja*). The upper margin of the iris in many rajaform fish is modified into a so-called *operculum pupillare* (Plate IIB-D) which descends over the pupil during light adaptation. The completely expanded operculum reduces the pupil to a series of stenopaic ("pinhole") apertures. According to Carrere (1922), the operculum is amuscular, and its mode of action is unknown. On the basis of fine structure, Kuchnow and Martin postulated that the operculum has no contractile mechanism and only a weakly dilatory mechanism, if any. They noted that if the top of a dilated eye is gently depressed the operculum abruptly descends, unconstricted, and rises again when pressure is released. Thus, according to Kuchnow and Martin, dilation results from relaxation of the iris sphincter and contraction of the iris dilator. When the spinchter contracts, opercular fibers that the authors call tonic allow the operculum to spring into shape, occluding the pupil.

Contraction of both pupils in response to illumination of only one eye is known as the consensual pupillary reflex. Such a reflex was long ago reported in *Raja* and then confirmed by Kuchnow and Martin. A consensual reflex is unexpected in skates since it does not occur in other elasmobranchs. The fine structure of the skate's iris did not elucidate the mechanism underlying this reflex.

Pupillary Activity—Pupillary kinetics of elasmobranchs have been reported by several authors (Gruber 1967, Kuchnow and Gilbert 1967, Kuchnow 1970, 1971). Gruber (1967) found that the pupil of *Negaprion* dilates fully in about 1h (Figure 6). Using infrared photography, he followed the course of pupillary dark adaptation after intense white light adaptation. At the onset of dark adaptation, the pupil was a vertical slit covering an area of about 10 mm^2. As the pupil dilated, the area doubled after 2 min in darkness and again after 5–7 min. A final doubling was observed between 40 and 55 min. Dilation was essentially complete after 1h. The maximum

increase in the area due to pupillary dilation was about one log unit, or tenfold.

According to Franz (quoted by Walls 1942), dogfish develop "mydriatic pupillary rigor," i.e., pupils first constrict, then dilate widely and remain dilated in an animal placed in continuous light for several days. Lemon sharks treated in this manner do not develop pupillary rigor (Gruber 1967).

Kuchnow and Gilbert (1967) measured pupillary responses of *Negaprion* to light and dark. After 25 min dark adaptation, the eye was illuminated with 1000 fc (1080 lx). The pupil rapidly constricted to a minimum diameter, then gradually adjusted to a steady state value some 5% larger. The entire process took about 4 min. They also followed pupillary changes during sunrise and sunset, noting that the greatest dilation (twofold) occurs as light falls between 10^{-3} and 10^{-5} fc (lx); the greatest pupillary constriction upon light adaptation occurs at 10^2 and 10^4 fc (lx). Differential rate of change at different illumination levels suggested separate mechanisms, which agreed with the morphology and early physiological studies of Young (1933) and von Studnitz (1933).

Kuchnow (1970) investigated details of pupillary activity in the diurnal *Mustelus* and nocturnal *Scyliorhinus*. *Scyliorhinus* was 2.5 times more sensitive (i.e., less light was required for pupillary constriction) than *Mustelus*. Response to light in both species was graded; the pupil of *Scyliorhinus* constricted to 18% after 1 min light adaptation and 5% of its dilated size after 5 min. The pupil of the *Mustelus* reacted differently: a drop to 18% after 2 min was followed by redilation to 20% after 5 min. The rate of pupillary constriction in *Scyliorhinus* shifted in the mid-range of light intensities. Kuchnow suggested that this shift signaled changeover from rod- to cone-control of a retinally mediated pupillary reflex. However, the diurnal *Mustelus*, known to possess retinal cones (Stell and Witkovsky 1973*b*), did not shift in sensitivity.

Pupillary activity of both species under monochromatic lights yielded an action spectrum similar to that of rhodopsin (Figure 7). Thus the melanin in the iris muscles cannot be the receptive pigment responsible for independent pupillary activity as suggested by Franz (1931). A rhodopsin-like pigment must therefore be present in the iris tissues.

In a final paper, Kuchnow (1971) photographed pupillary activity in 13 elasmobranchs. Nocturnal and diurnal species had mobile pupils; the deep-sea sharks *Oxynotus* and *Apristurus* had fixed pupils. Dilation in all other species was slow, typically taking 30 min. Constriction in Rajaformes ordinarily required about 5 min. Pupillary constriction in bright light was comparatively slower—about 15 min for the five species tested. Most unexpected was the rapid dilation observed in *Carcharhinus galapagensis* (Plate IA shows a closely related species, *C. limbatus*). This shark fully dilated in only 1 min, faster even than constriction, which required 2 min. The observation was made on shipboard and may represent an abnormal condition since dilation is apparently much faster in this species than in any known elasmobranch including other carcharhinids. However, Sivak and Gilbert (1977) have recently observed rapid dilation in the sandbar shark, *C. milberti*. None of
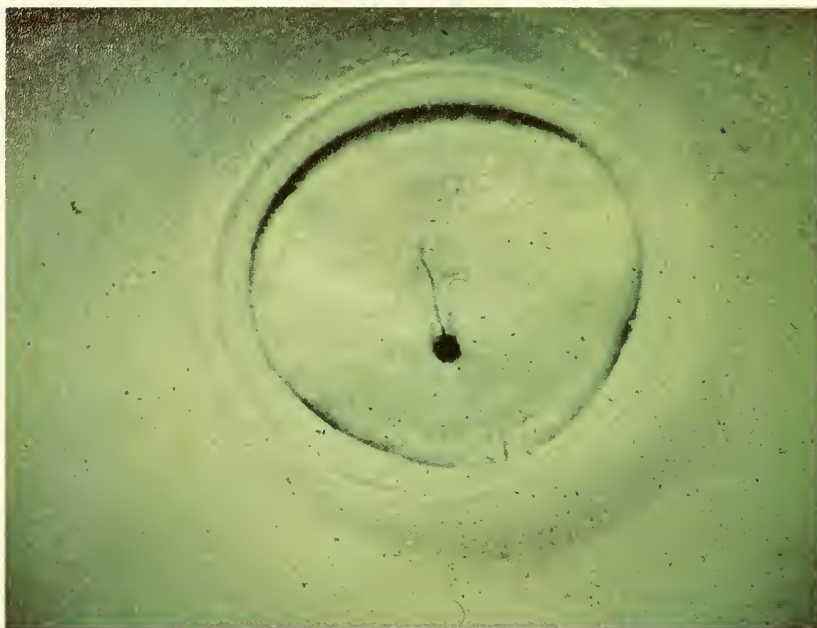
Light-adapted eye of the blacktip shark, *Carcharhinus limatus* (Valenciennes)—Carcharhinidae—showing vertical slit pupil entirely contracted except for the apparent stenopaic aperture or pinhole at the bottom. Plate No. IA
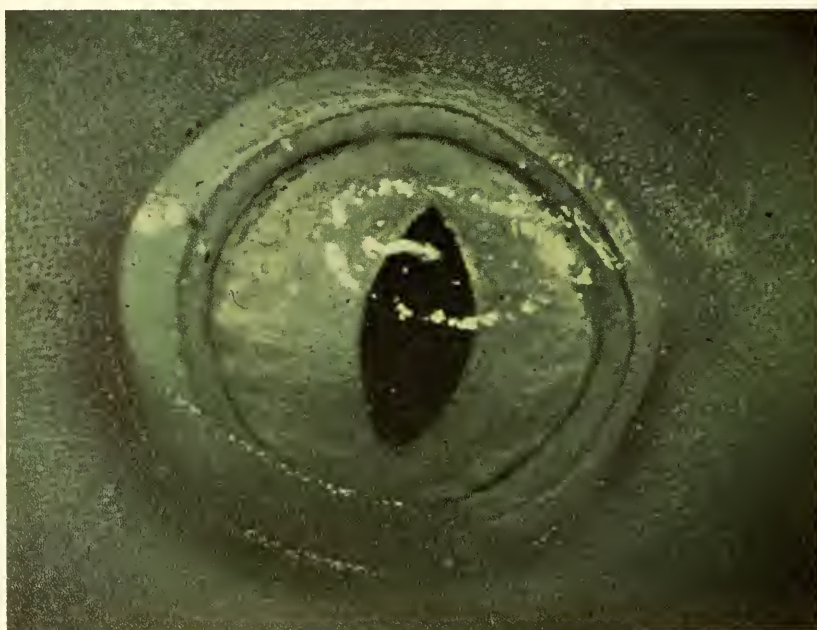
Partially dark-adapted eye of the same blacktip shark with the iris dilated to a circular form. Plate No. IB

Light-adapted eye of the bonnethead shark, *Sphyrna tiburo* (Linnaeus)—Sphyrnidae. In contrast to the blacktip, this shark has a horizontal slit pupil. Plate No. IC
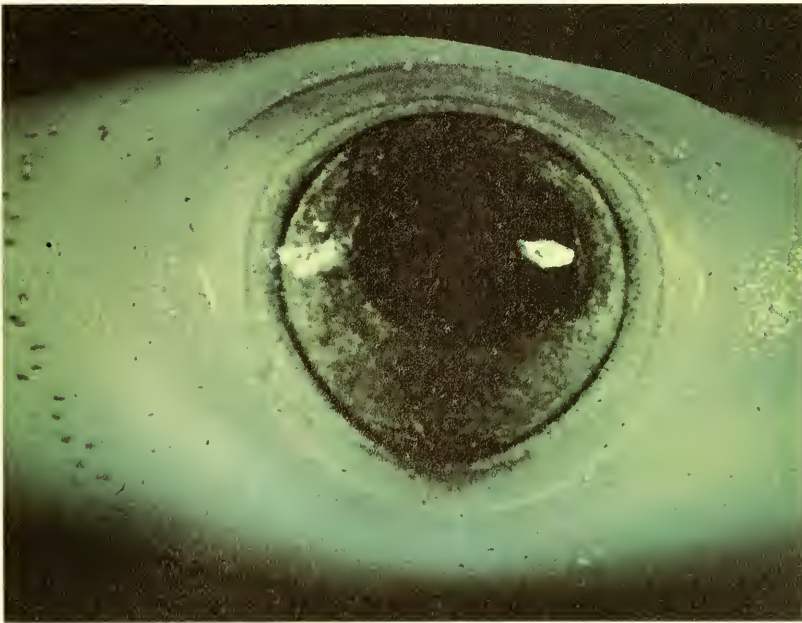
Partially dilated iris of the bonnethead shark. The pupil rounds up as it expands during dark adaptation. Plate No. ID

Light-adapted eye of the nurse shark, *Ginglymostoma cirratum* (Bonnaterre)—Orectolo-
bidae. Orientation of the slit pupil in this shark is oblique. Plate No. IIA

Partially dark-adapted eye of the cownose ray *Rhinoptera bonasas* (Mitchell)—Mylio-
batidae. Unlike most batoids pupil shape in this family with no trace of an *operculum
pupillare*. Plate No. IIB

Photo courtesy of Constance Warner

Light-adapted eye of the smalltooth sawfish, *Pristis pectinata* (Latham)—Pristidae. The upper margin of the iris is developed into a robust digitiform *operculum pupillare* which descends during light adaptation as shown. Plate No. IIC

Photo courtesy of Constance Warner

Light-adapted eye of the thorny skate, *Raja radiata* (Donovan)—Rajidae—showing the well-developed *operculum pupillare*. The multiple fingers form a series of stenopaic apertures during light adaptation, which provide nearly infinite depth of field combined with ray selection. Plate No. IID

Figure 6   Pupillary dilation in *Negaprion brevirostris* revealed by infrared photography. The shark was intensely light-adapted, then placed in darkness and photographed after various intervals. During the 60 min of dark adaptation the pupil increased tenfold in area and changed shape from slit to circular. This shark's iris contracted somewhat less than usual. Left number is min in darkness; right number is pupillary area in mm. (Taken from Gruber 1967. P.W. Gilbert, R.F. Mathewson, and D.P. Rall, eds. © 1967 by permission of the Johns Hopkins University Press.)

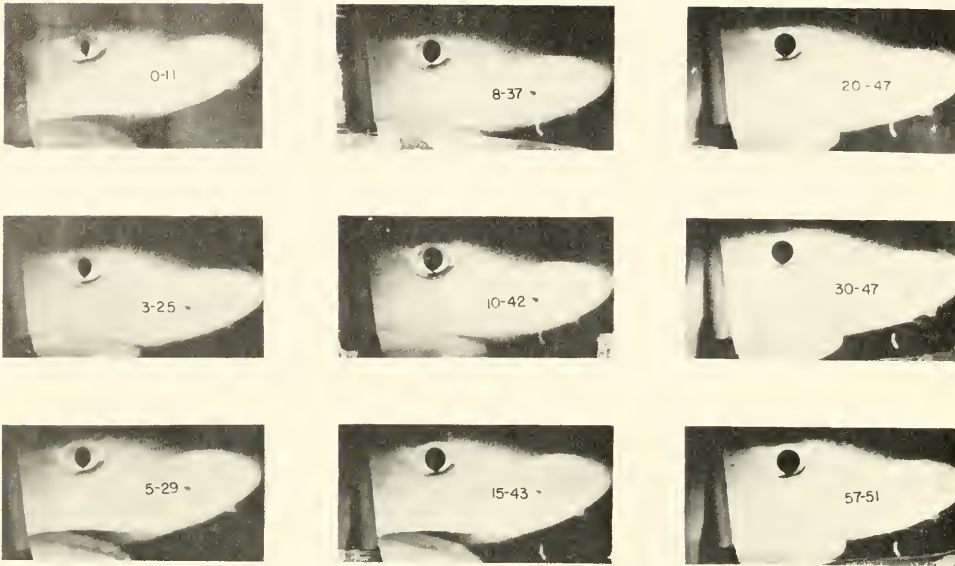the species tested, including Rajaformes (*Dasyatis* and *Myliobatus*), displayed a consensual reflex.

Pupillary control mechanisms were also investigated by Kuchnow, using electrical, pharmacological, and photic stimulation combined with denervation and extirpation. Injection of prostigmine speeded dilation, while d-tubocurarine inhibited it. Similarly, sectioning of the *occulomotorius* also inhibited dilation and speeded constriction in light. The illuminated eye dilated upon electrical stimulation of the *occulomotorius*. Illumination of the retina alone (1-mm spot through the pupil) caused rapid and complete pupillary constriction, while illumination of the iris with this spot produced only local constriction. From these results, Kuchnow suggested that pupillary size is the result of two opposing forces: (1) the action of both light and neural signals on the sphincter and (2) the tonus of the dilator. It was originally thought that during dilation the iris was an independent effector only (Young 1933), but Kuchnow has demonstrated a neural component in the dilator system. Finally, results with spots of light suggest an iris-retina reflex. Kuchnow thus proposed that the iris of diurnal sharks constricts rapidly because of greater influence of nervous mechanisms. Nocturnal sharks are intermediate and Rajaformes are slowest, probably reflecting a decrease in importance or absence of nervous control.
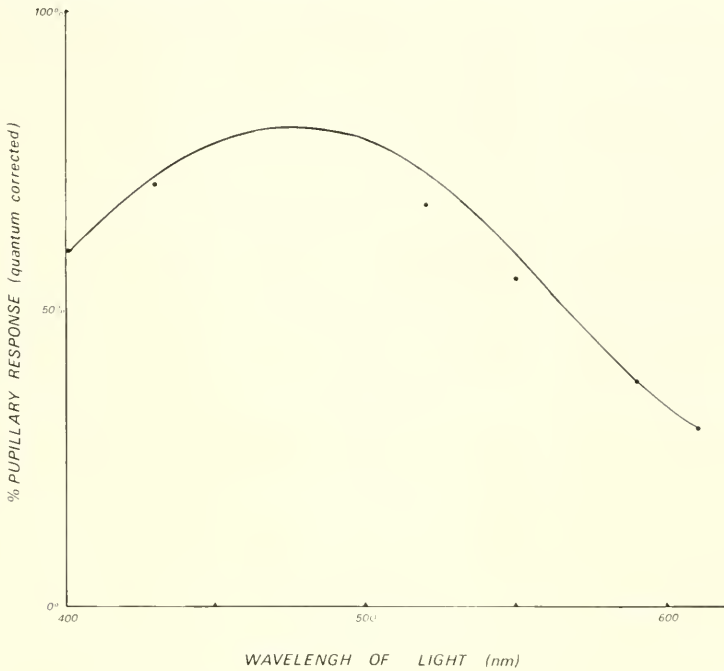
Figure 7   Pupillary dilation in *Scyliorhinus canicula*. Kuchnow's (1970) data were corrected for quantal absorption and replotted. The resultant curve resembles the absorption spectrum of the visual pigment of this species.

Ciliary Body—The remaining studies on the ciliary zone of elasmobranchs are concerned with production, composition, and drainage of aqueous humor. In many vertebrates aqueous humor is produced by an active process in the epithelium of the ciliary body and secreted into the posterior chamber of the eye. From there it flows to the anterior chamber via the pupil, to be drained away at the iridocorneal angle. The aqueous has important optical, nutritive, and excretory functions in the largely avascular anterior segment of the eye, but perhaps most important are its hydromechanical properties and their relation to the optics of image formation.

Rate of aqueous production and resistance to drainage are the principal factors determining intraocular pressure (IOP). Changes in these factors leading to increased IOP produce the condition know as glaucoma, one of the leading causes of blindness in man. Thus intensive study has aimed at understanding factors affecting IOP in the vertebrate eye. As already mentioned, similarities between the mammalian and elasmobranch ciliary body make the shark an ideal model for the study of basic mechanisms of aqueous production and drainage.

Duke-Elder (1932) claimed that aqueous humor was merely a dialysate of the plasma, presumably derived from the ciliary vascular system. However,

comparison of ascorbate concentration in human plasma and aqueous showed that ultrafiltration alone could not account for the composition found.

Aqueous Humor, Composition—In a noteworthy study, Doolittle and Stone (1960) determined the composition and measured osmotic pressure of the aqueous humor in *Mustelus*. Table 1 shows their major findings. Of 14 components common to plasma and aqueous, an excess in the aqueous of ascorbic acid (3×), sulfate (1.4×), and bicarbonate (2.5×) was measured. However, the total osmotic pressure of aqueous humor was about 25 milliosmoles lower than that of the plasma, due primarily to urea and trimethylamine oxide. This suggests that water passes into the eye against the gradient.[*] Doolittle et al. thought that the pattern of formation of aqueous humor in the shark was similar to that in mammals, citing as evidence ciliary body anatomy, plasma : aqueous distribution ratios, occurrence of carbonic anhydrase, and aqueous turnover rate (1% per min).

Davson and Grant (1960) confirmed that the aqueous of *Mustelus* is hyposmolar to the plasma, but the difference was somewhat lower than the figure given by Doolittle and Stone. They also measured an intraocular pressure of 7.8 mm Hg in *Mustelus;* the IOP of man is 15.5 mm Hg (Davson 1969). Stone et al. (1960) agreed that the aqueous of *Mustelus* was

Table 1. Normal electrolyte composition of elasmobranch aqueous humor.

Constituent	Smooth dogfish[*] *M. canis*		Spiny dogfish[†] *S. acanthias*	
	mM/kgH$_2$O	Ratio aqueous to plasma	mM/kgH$_2$O	Ratio aqueous to plasma
Urea	320	0.94	350	1.0
TMAO	85	0.88	‡ 100	1.0
Sodium	279	0.97	279	1.02
Potassium	7	0.88	5.5	1.25
Chloride	256	0.95	253	1.0
Bicarbonate	15	2.5	8.5	1.09
Osmolarity	935	0.97[‖]	979	1.0[¶]
pH	7.86	1.07	7.65	1.0

[*]Doolittle and Stone (1960).
[†]Maren (1973).
[‡]Inferred value.
[‖]Hypo-osmotic.
[¶] Isosmotic.

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[*]It should be noted that the finding of aqueous humor with an osmolarity lower than that of plasma is difficult to understand, since a mechanism for passage of water into the eye against the gradient has not been shown.

hyposmolar to the plasma, and obtained experimental evidence that environmental water does not pass through the cornea into the eye. This agrees with the results of Edelhauser (1968) discussed previously. Finally, they confirmed the presence of high levels of carbonic anhydrase in the ciliary body.

Maren (1962a, 1962b, 1973) and Maren et al. (1975) analyzed the aqueous humor of *Squalus*. Differences in the properties of aqueous humor between *Squalus* and *Mustelus* appeared to be significant (Table 1). As in mammals, Maren found the aqueous of *Squalus* to be iso-osmotic to plasma. In nearly all electrolytes the distribution ratios of *Squalus* exceeded those of *Mustelus*. Differences in rate of aqueous production and carbonic anhydrase activity were also reported. These differences have surprised several authors (Maren 1973, Jampol and Forrest 1972, and Zadunaisky 1973) because of the supposed taxonomic closeness of these two species. In fact, these elasmobranchs are phylogenetically dissimilar, being on ends of evolutionary lines that separated as early as the Jurassic (Schaeffer 1967). Thus they are distinguished at the ordinal level in a way similar to the primate and rabbit—species that differ considerably in their aqueous mechanisms (Maren 1973).

Aqueous Humor, Formation—Jampol and Forest (1972) investigated the mechanism and site of aqueous formation in *Squalus*. In man, aqueous is almost certainly secreted from the nonpigmented cuboidal epithelium of the ciliary body (Davson 1969). The ciliary body of elasmobranchs is quite prominent—especially compared with that of teleosts—and similar in many respects to its mammalian counterpart, including the presence of nonpigmented cuboidal epithelium. Maren (1962b) had already suggested an active transport mechanism based upon the secretory appearance of cellular elements in the ciliary body, the presence of carbonic anhydrase, and the effect of carbonic anhydrase inhibitors on production and composition of aqueous. Doolittle and Stone (1960) also speculated on active processes, as described previously. Jampol and Forrest analyzed ciliary tissue for the enzyme adenosine triphosphatase, which in the presence of Mg^+, Na^+, and K^+ catalyzes the reaction

$$ATP \leftrightarrows ADP + Pi.$$

This reaction provides high-energy bonds to run "sodium pumps" in a wide variety of tissues. The occurrence of Na-K-ATPase in the ciliary body was demonstrated by Jampol and Forrest and is further indirect evidence of active secretion of aqueous in the shark. The authors compared Na-K-ATPase activity in various ciliary structures of *Squalus*, finding the highest activity (greater than 5× compared with the iris) in the ciliary body proper. Thus, it seems likely that the formation of aqueous takes place in the ciliary body and utilizes active transport.

Aqueous Humor, Dynamics—Chemistry and dynamics of aqueous in *Squalus* were further studied by Maren et al. (1975) to determine whether a constant phylogenetic pattern underlies the physiology of aqueous formation in vertebrates. Anatomic and enzymatic similarities between sharks and

mammals had already been demonstrated, suggesting analogous functional patterns. Maren et al. confirmed the aqueous volume at 0.25 ml with a turnover rate of 0.4 ml/h, about half the mammalian value. The vitreous volume was estimated at 3.0 ml. Movement of Na^+ and Cl^- and HCO_3^- from plasma to aqueous was followed: it appeared that accumulation of Na^+ and Cl^- was diffusion limited, while HCO_3^- formation and accession was different (i.e., much faster).

Maren's data on bicarbonate accumulation in the shark and Kinsey and Reddy's (1959) data on the rabbit are similar. Ion exchange between aqueous and vitreous was much greater than between plasma and aqueous, suggesting that the vitreous is an important ionic outflow pathway in this animal. Maren et al. deny the existence of a canal of Schlemm or aqueous veins in *Squalus*, thus increasing the importance of the vitreous pathway. However, studies on *Squalus* by Tripathi (1974) confirmed the finding by Rochon-Duvigneaud (1943) of a sinus of Fontana—the analog of Schlemm's canal in lower vertebrates. Tripathi also presented evidence for a conventional outflow pathway similar to that of higher mammals.

The shallow anterior chamber of elasmobranchs is bounded posteriorly by the iris, which is bowed forward by the protruding lens. Thus, the angle formed by the meeting of the cornea and iris is narrow and may extend back nearly to the *ora serrata*. At the narrowest part of the iridocorneal angle the so-called annular ligament is absent. However, ventrally the angle is wide and filled with a meshwork of collagen fibrils and microfibrillar elements corresponding to the annular ligament. This meshwork supports a large endothelium-lined sinus probably similar in function to Schlemm's canal in mammals. Endothelial cells on the inner wall of this sinus contain giant vacuoles about 10 μm in diameter, comparable to those seen in mammals. Preliminary studies suggest that this endothelium plays a significant role in bulk outflow of aqueous humor. The elasmobranch condition fits nicely into Tripathi's (1971) theory of vacuolar, transcellular aqueous outflow. Tripathi (1974) imagines the initial state of outflow as macropinnocytosis, the endothelial cells ingesting then transporting aqueous from the annular ligament into the sinus.

The Ocular Lens

The vertebrate pattern of development and structure of the ocular lens is remarkably consistent (Clayton 1974). Two obvious variations are found: the system of cellular sutures occurring in most vertebrates is absent in cyclostomes and a few reptiles and, depending on habitat and mode of accommodation, the lens is either spherical and inelastic or flattened and resilient. The cornea, principal refractor in the aerial eye, is optically absent underwater. Thus, most aquatic vertebrates, including elasmobranchs, possess a voluminous lens.

The elasmobranch lens is slightly compressed on the optical axis and thus subspherical or lenticular in shape (Figure 2). It has a relatively simple sutural complex consisting of a single line running vertically in the anterior

and horizontally in the posterior pole of the lens (Duke-Elder 1958, Prince 1956).

The lens of elasmobranchs has lately received much attention, with two central themes: characterization of the crystallin protein fractions and immunochemistry. The former relates to clinical interest in the origin and treatment of cataract, and the latter primarily with systematics and phylogeny of the vertebrates. However, the subjects are interrelated since it is the evolutionarily conservative antigenic proteins that are responsible for lens cataract.

Lens Crystallins—The ocular lens is 65% water and about 35% structural protein (Lerman 1969). Mörner (1894) long ago showed that lens proteins could be separated into soluble crystallin and insoluble albuminoid fractions. Three types of crystallin were identified and assigned the Greek letters α, β and γ on the basis of molecular weight, number of subunits, and other physicochemical characteristics. A fourth species, Delta crystallin, was later isolated (Rabaey 1962). Alpha, β, and γ crystallin are heteropolymers, α having the greatest number of subunits. Gamma crystallin is a monomer (Clayton 1974). These proteins play a major role in refraction and transmission of light, which is the main function of the lens. Trokel (1962) suggested that the spatial orderliness of intact protein fibers confers transparency on the lens. The high concentration of refractile protein gives the lens its overall high refractive index (Clayton 1974).

Insoluble protein—Changes in relative concentration of crystallin are associated with a progressive increase of insoluble albuminoid throughout life. In man, normal increase in albuminoid during aging is reflected in loss of lens elasticity, leading to presbyopia in middle age. Abnormal increase leads to nuclear cataract formation. Lerman (1970) presented a summary of his earlier studies (Lerman et al. 1968, Lerman 1969) on ontogenetic protein changes in the dogfish. He reported that during aging soluble γ protein in the lens of the "dogfish" (species not given) progressively changes to insoluble albuminoid by polymerization and formation of S-S linkages. This situation is similar in the rat but quite different in the lens of man: in humans, the insoluble protein is derived mainly from β crystallin. Lerman in addition listed the amino acid content of α, β, γ, and albuminoid fractions of the dogfish lens.

Mehta and Lerman (1970) isolated β and γ crystallin from the insoluble fraction of the dogfish lens. Alpha may also be present, but it is albuminoid perhaps "masked" by incorporation into aggregates.

Zigman et al. (1970) measured a 240-fold increase in weight of total insoluble lens protein (TIP) beween young "dogfish" sharks 60 cm in total length and older animals of 150 cm. Lens weight in the experimental animals increased by only tenfold. Thus the lens of the young dogfish contained 1% TIP by weight while that of the adults contained 25% TIP. Most of the shift was toward urea-soluble albuminoid. Again, ontogenetic changes in the lenses of both rat and dogfish were remarkably similar.

Soluble protein—Methodology in the following studies is aimed mainly at separating and classifying structural (nonenzymatic) proteins of the lens. This requires one or a combination of techniques for arriving at molecular weight, charge, or antigenic specificity of the protein in question. However, many techniques with varying relationship to one another are available. For example, there are no less than six independent immunoassays. Thus, part of the conflicting results reported here may be due to varying methodology rather than to species differences. Unfortunately, extraction and preparation of lens protein has not yet been standardized. In addition, it is frequently difficult to determine the age of a fish, an important consideration since, as just mentioned, protein composition changes in ontogeny. Thus, further disparities of results from different research groups can be expected (Clayton 1974).

Bon and his coworkers (Bon 1958, Bon et al. 1964, 1966, 1968) have analyzed soluble proteins of the ocular lens by a variety of techniques. Using agar electrophoresis, Bon (1958) found a remarkable similarity between α and β crystallin of the adult shark (*Scyliorhinus*) and the newborn calf. He reported that α crystallin of *Scyliorhinus* changes little during ontogeny, while β crystallin continuously increases throughout early life. This is similar to the pattern in higher vertebrates. Bon further speculated on genetic mechanisms of crystallin formation.

Bon et al. (1964) characterized the soluble lens proteins of five elasmobranch species, all adults. Pronounced α and β groups were noted, as well as a series of low electrophoretic peaks representing γ and ∂ proteins. This was apparently the first observation of ∂ crystallin in the fish lens. It is not clear, however, if Bon's ∂ crystallin corresponds to Rabaey's (1962) so-called first important soluble component (FISC) of the bird, which was later called ∂ crystallin. Indeed, Bon felt that Rabaey's ∂ crystallin was identical to vertebrate β crystallin.

Bon correctly noted the phylogenetic disparity between the sharks he investigated, pointing out that spiny dogfish and blue shark represent separate ordinal levels. Yet the lens proteins of these two sharks were structurally more closely related than the proteins of *Scyliorhinus* (taxonomically close to the blue shark) or *Raja* (relatively close to the spiny dogfish). The authors thus suggested an environmental effect on protein elaboration since *Scyliorhinus* and *Raja* are benthic while *Squalus* and *Prionace* are pelagic.

Bon et al. (1968) further characterized ∂ crystallin in adults of four elasmobranch species. Basically different gel filtration and sedimentation patterns between teleosts and elasmobranchs, especially Squaliformes and Rajaformes, were found. Surprisingly, the elasmobranch pattern more closely resembled that of mammals than that of teleosts. However, Rabaey (1965a) disagreed, finding little difference between the protein patterns of sharks and bony fish.

Cobb et al. (1968) criticized the usual methods of identifying lens crystallins, claiming that the best methods rely on the specification of charge, electrophoretic mobility, size, shape, and distribution of charge, combined with free electrophoresis, zone electrophoresis, and sedimentation analyses.

Thus, they studied several teleosts and the bonnethead shark, *Sphyrna tiburo*, reporting the absence of α crystallin in bony fish as had been previously suggested by Cobb and Koenig (1968). However, others (i.e, Rabaey 1965*b*) have identified small amounts of α crystallin in the fish lens. In fact, Mehta and Lerman (1971) investigated the properties of dogfish (*Mustelus*) α crystallin. Electrophoretic mobility of shark α was significantly slower than that of mammals and one bird. In addition, the amino acid pattern of dogfish α was quite different and did not cross–react in an immune assay with any other species tested. Therefore, dogfish α crystallin is fundamentally different from that of higher vertebrates.

Cobb et al. (1968) were eventually able to isolate a component in the lens of *Sphyrna* with a sedimentation coefficient of mammalian α crystallin. The authors reported that about 70% of the native soluble protein in the shark lens exists as a low molecular weight fraction equivalent to γ crystallin. They also found an intermediate weight fraction apparently corresponding to β crystallin.

Analyses of lens crystallins have been used as tools in both population and taxonomic studies. These investigations are based on occurrence of species-specific lens proteins. However, at least two nongenetic sources of variation exist: protein changes through ontogeny and changes occurring between death of the animal and protein extraction.

Peterson and Smith (1969) investigated intraspecific variation in the ocular lens proteins of the sandbar shark, *Carcharhinus milberti*. Expected ontogenetic changes were shown by comparison of adult lens proteins to those of near-term fetal sharks. The adults were collected from different islands in the Hawaiian chain and possibly represent separate populations. The individual protein pictures were quantitatively and qualitatively heterogeneous: no less than 10 different electrophoretic patterns were described. The authors related the different patterns to different groups of sharks, suggesting that this is a practical method of identifying separate breeding populations.

Lenses of young mammals cooled to less than 10°C become reversibly opaque due to precipitation of cold-precipitable protein fraction (CPP) (Lerman and Zigman 1965). Upon warming, the lens again becomes transparent. The importance of CPP relates to ontogenetic changes in the lens: along with the albuminoid fraction, the most marked changes in protein concentration occur in the CPP fraction. In mammals, CPP apparently consists of a single component with a sedimentation rate differing from that of all three crystallins. Normally, the phenomenon of cold cataract does not occur in the elasmobranch lens. However, Zigman et al. (1964) observed that treating mammalian lenses with 0.3 *M* urea tended to inhibit CPP formation. Reasoning that concentration of urea in elasmobranch tissues (0.25 *M*) might be a factor blocking formation of CPP, they extracted the water-soluble lens proteins of the dogfish, *Mustelus*, and removed the urea by dialysis. Cooling the dialysed preparation to below the freezing point of water precipitated a CPP fraction. Replacing the urea after warming again prevented CPP formation. As in the rat, dogfish CPP decreases markedly

from 17% to 5% during aging. Gel filtration of dogfish CPP revealed fractions of three crystallins, α and β contributing about 20% and γ the remainder. Dogfish CPP is thus similar in physicochemical properties to its mammalian counterpart. The authors suggested that urea retention is an environmentally tuned factor maintaining clarity at the lens at less than $10°$ C. However, one must inquire into factor(s) maintaining lens clarity in the majority of fish which do not retain urea but possess transparent lenses even at less than $0°$ C.

Calhoun and Koenig (1970) confirmed the presence of CPP in the dialysed lens of the hammerhead shark, *Sphyrna diplana.*

Immunochemistry—Perhaps the most interesting work on lens proteins has been the use of their antigenic properties to estimate the evolutionary history of the vertebrates. Basic to these studies is organ specificity first descirbed by Uhlenhuth (1903). He injected rabbits with small amounts of bovine lens proteins, which resulted in antibody production specific to the injected proteins. Serum antibodies from such "immunized" rabbits reacted not only with bovine lens proteins but also with lens material from many other vertebrates. This Uhlenhuth called organ specificity, in contrast to species specificity seen for example in blood serum proteins which cross-react within a few closely related species (Manski et al. 1965). This means that the blood proteins of unrelated animals are quite different, while the lens proteins can be identical. In addition, lens antigens (proteins) are considered to be restricted to the lens. This is known as tissue specificity, although not all authors so distinguish between species, organ, and tissue specificity (Waley 1969).

Organ specificity is taken to mean that lens proteins shared by two or more modern vertebrate classes must have originated in a common ancestor. This statement forms the basis of the study of vertebrate phylogeny on a molecular level (Manski et al. 1965). Early ontogenetic isolation from the rest of the body combined with specific optical constraints and phylogenetically rapid perfection may explain the apparently slower and conservative evolution of lens proteins.

The concept and supporting data of tissue specificity have recently been criticized (Clayton et al. 1968). The authors listed 28 publications (1944–1965) in which antisera prepared against lens crystallins reacted not only with eye tissue but also with extraocular tissue such as liver, kidney, and muscle. Clayton et al. do not distinguish between organ and tissue specificity, using both terms synonymously in the text. The result of their study confirms that the lens contains a mixture of proteins with perhaps a few specific, while the remainder are widely distributed throughout the body. Thus, tissue specificity is viewed as a tendency toward a specific combination and concentration of antigens rather than a unique group of proteins restricted to the lens.

Manski and his colleagues (Manski and Halbert 1965, Manski et al. 1965, 1967*a*, 1967*b*, 1967*c*) have investigated in detail the antigenic relationships of lens proteins among a host of vertebrate species. Their elasmobranch work

has been confined to *Negaprion*, *Carcharhinus*, and *Scyliorhinus*. Typically, fresh frozen lens material was homogenized with Freund et al.'s (1937) adjuvant and injected into adult chinchilla rabbits for periods up to 2 years. Antibodies, concentrated by precipitating out the globulin fraction of the antiserum, were tested with lens antigens by the methods of immunodiffusion (Ouchterlony 1949) and immunoelectrophoresis (Scheidegger, 1955).

Results on sharks revealed first that testing antidogfish sera with either dogfish, bull shark, or lemon shark lens homogenates gave reactions that were indistinguishable from one another. This shows that all sharks and probably all cartilaginous fish possess the same lens antigens. No immune reaction was observed when antishark serum was tested against squid or lobster lens antigens. Thus, Manski et al. demonstrated that analogous lens tissues are biochemically unrelated, supporting the notion of convergent evolution of the invertebrate and vertebrate eye. Antishark sera tested against lenses from all other vertebrate classes reacted to some extent. Therefore all classes of vertebrates possess ancient lens proteins, at least one of which is shared with early cartilaginous fishes.

The full significance of this research is beyond the scope of this review, but one crucial test can be discussed. To follow the evolution of crystallins through the vertebrate phylogenetic series, Manski et al. absorbed lens antigens in antisera from one group and tested with another. For example, if they wished to determine what antigens were shared by, say, sharks and teleosts but evolved after contribution of antigens by the cyclostomes, standard antishark serum was prepared. To this, cyclostome lens homogenate was added, precipitating out all antigens specific to that group. Such a "reagent serum" could now be tested with lens homogenates of many other vertebrates. This Manski termed "systematic absorption" of antisera. Results of these experiments demonstrated that at least four primitive crystallins derived from the agnatha were retained by elasmobranchs and all other vertebrates. At least six others that originated after the agnatha were transferred to higher vertebrates. Alpha and β crystallins remained relatively unchanged during transfer through the vertebrate series, while γ crystallin did not transfer well. Analyses of all test combinations led to a provisional molecular phylogeny for the fishes: ". . . agnatha were the first vertebrates to appear. From the agnatha descended all jawed fish . . . one branch led to the primitive choanicthyes (lung fishes), the other led to some other form of primitive jawed fish ancestral to the bony actinopterygii (represented today by the bichir) and to the . . . modern sharks." (Manski et al. 1967c)

An interpretive problem with antigens studies is that protein fractions with different electrophoretic mobility display identical immunological properties. Thus, Rabaey (1965a) warned that, for phylogenetic studies, it is not desirable to analyze a single body fluid or tissue extract.

Lens Metabolism—Several studies on lens metabolism in the dogfish have been published by Lerman and his coworkers. Lerman's major concern was the type of RNA found in the lens and its role in aging. Although the experimental species was not identified in any of the cited research, we

assume, based on Lerman's early work, that the "dogfish" was *Mustelus canis.*

Lerman et al. (1962) described three types of RNA from the dogfish lens: insoluble or albuminoid, not found in any other body tissues; soluble or SRNA; and microsomal. Little RNAase activity was reported. The pattern of aging relative to changes in RNA was similar in dogfish, skate (species not given), rat, and rabbit: SRNA and microsomal RNA remained unchanged, while insoluble RNA increased in older animals. Carbohydrate metabolism, however, appeared to be very much different in the shark. Intermediary metabolism in the lens of mammals consists of the well-known pentose phosphate pathway, glycolysis, and the less universal sorbitol pathway (van Heyningen 1969). In the shark, glycolysis is the major pathway of lens glucose oxidation throughout life. In contrast, the pentose phosphate pathway is most active in young rats but diminishes in importance during aging. Paradoxically, the RNA pattern in carbohydrate metabolism of the skate lens was more closely akin to that of the rat than that of the dogfish.

Lerman and Fontaine (1962) confirmed the similarity of dogfish and rat RNA but reported that insoluble RNA increases tenfold in the dogfish during aging and only twofold in the rat. In addition, labeled (C^{14}) leucine and P^{32} incorporation studies indicated a marked decrease in turnover of albuminoid RNA during aging.

Lerman et al. (1963) determined composition and nucleotide ratios of lens RNA in rat and dogfish by paper chromatography and subsequent spectrophotometric analysis. Results again indicated a close similarity between rat and dogfish: purines (adenylic and guanylic acid) were present at about 30 moles percent while pyrimidines (cytidylic and uridylic acid) were found to be 20 moles percent. The composition of lens and kidney RNA was also very similar.

Lerman et al. (1965) confirmed the RNA base ratios given above and reported that the albuminoid RNA molecules were relatively small, with a molecular weight of less than 50,000. The albuminoid RNA apparently derived from soluble RNA. Results of a so-called pulse labeling experiment involving uptake of C^{14} uracil in an effort to identify messenger RNA were inconclusive. Uptake in any case was exceedingly slow—10^{-6} nmol/h. Metabolic inhibitors (ouabain, cyanide) apparently facilitated passage of labeled uracil through the lens capsule. Finally, the authors remarked on the similarity of dogfish and mammalian lenses, in that both have a single layer of subcapsular epithelium along the anterior lens surface extending slightly beyond both equators.

The Retina

In strictly visual terms, the retina, an embryological extension of the brain, is the most important structure of the vertebrate eye. The other ocular components can be thought of as accessory structures. It is in this nearly transparent tissue that transduction of a photic stimulus into an electrochemical signal takes place. How that signal is produced and processed and in what

structures this activity occurs form the discipline of correlative retinal physiology (Stell 1972a). This marriage of biochemistry, physiology, and morphology has produced, in the last 10-15 years, what must be regarded as astounding progress in the field of visual processes. This progress has no doubt been aided by the general advance in biological technology, but that cannot be the whole story. Generous research support combined with the dedication of a number of brilliant workers and development of some highly analytical methods (i.e., microelectrode technique, radioautography, single-cell microspectrophotometry, freeze-etch methods, etc.) have added to the unparalleled growth of our understanding of the retina. The elasmobranchs have become increasingly important experimental subjects during this growth period, as evidenced by the more than 60 publications on the retina and vision of these fishes between 1960 and 1975.

Retinal Anatomy—Several excellent and comprehensive descriptions of retinal organization in the vertebrates are available (Dowling 1970, Dunn 1973, Rodieck 1973, and Stell 1972a) and the reader is referred to these for details. For reference, however, a brief description of the generalized verte-brate retina will be given.

The sensory retina is a highly ordered tissue arranged into three cellular and two synaptic layers (Dowling 1970). The scleral border of the retina is comprised of a layer of nutritive cuboidal cells, the retinal epithelium.

The epithelium is separated from the choroid by a basal lamina, Bruch's membrane. In man, Bruch's membrane is a complex pentalammelar structure composed of connective tissue (Rodieck 1973). The retinal epithelium, in addition to nutritive function, is active in visual pigment regeneration and renewal of the outer segments of rod photoreceptors. Fine structure of the elasmobranch pigment epithelium is poorly known; however, one note-worthy feature is that it lacks the characteristic melanin pigment granules found in other vertebrates (Müller 1856).

The most distal of the three cellular layers comprising the vertebrate retina is the receptor or bacillary layer containing the rods and cones. This means that light must pass entirely through the retina before impinging on the receptors.

The majority of synapses between retinal neurons are confined to two synaptic or plexiform layers. The outer plexiform layer is the synaptic site of receptor terminals (rod spherules and cone pedicles) and the dendrites of horizontal and bipolar cells, both of which comprise the inner nuclear layer along with the perikarya (cell bodies) of the amacrine cells. Amacrine and bipolar cells contact ganglion cells in the inner plexiform layer and the ganglion cell bodies along with their axons form the innermost (vitread) optic nerve and ganglion cell layers.

This basic organization was well known by the 19th century histologists, especially from the work of Ramon y Cajal (1893, etc.). One other major group of cells, known as Müller or radial fibers, traverses the retina vertically from receptor to ganglion cell layer; they are glial elements which provide support for the neurons.

Receptor Cells—Photoreceptors of elasmobranchs have been continuously investigated since as early as 1866 (by Schultze). Recently, however, a basic change in our understanding of the elasmobranch retinal receptor types and correlated function has occurred (Figure 8).

Hannover (1840) and later Müller (1851) and Schultze (1866) distinguished two types of vertebrate photoreceptors—the rods and cones. A formulation known as the Duplexity Theory of vision which assigns separate visual functions to each receptor type has come down from these original findings. The basic assumption is that functioning of the retina in dim light (scotopic conditions) is mediated by the rod system, while cones take over
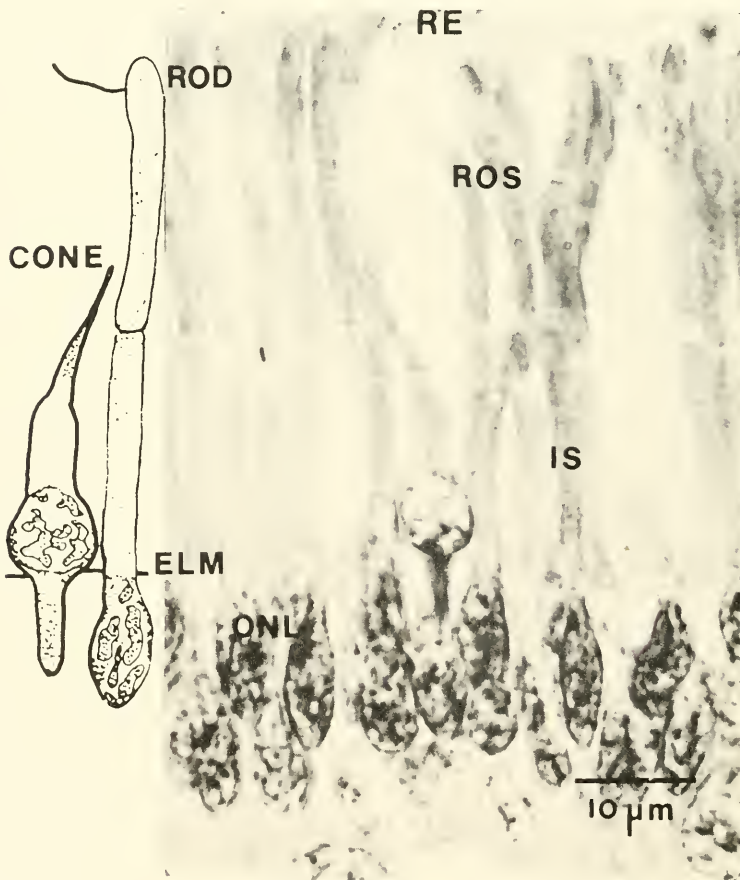

Figure 8 Light micrograph of the photoreceptors of *Carcharodon carcharias*. The maximum rod-cone ratio in this species was 4:1. ELM = external limiting membrane; HC = horizontal cell; IS = inner segment; ONL = outer nuclear layer; RE = retinal epithelium; ROS = rod outer segment. (From Gruber et al. (1975). Reprinted from the *Bulletin of Marine Science* by permission.)

during daylight (photopic conditions). Such a division of labor is expected and reasonable in view of the day-night dichotomy of the photic environment, and, all else being equal, visual systems should adapt to this duplex ecological factor. This is precisely what has occurred, if we accept the Duplexity Theory.

Rather detailed accounts of the evidence and inconsistencies of the Duplexity Theory are given by Pedler (1965), Willmer (1965), Graham (1965), Cohen (1972), and Crescitelli (1972). The important point for this discussion is that until recently the elasmobranch retina was said to be cone-free, except in one or two species. Actually there were several early reports of duplex elasmobranch retinas, but these were not effective in changing the viewpoint expressed first by Schultze (1866); later by Verrier (1930), Walls (1942), Rochon-Duvigneaud (1943), Prince (1956), Duke-Elder (1958), and Gilbert (1963); and more recently by Wolken (1975), namely, that sharks possess a pure rod retina entirely devoid of cones and are thus extremely specialized for nocturnal vision. Since 1963, when Gruber et al. presented unequivocal histological evidence of cone photoreceptors in the retina of the lemon shark, *Negaprion*, many authors have confirmed this general finding in all taxonomic orders of elasmobranchs (Table 2). This finding is all the more reasonable in view of Cohen's (1972) statement that electron microscopists have failed to find a retina that is free of the type of output terminal—the pedicle—characteristic of cones. The one exception appears to be the pure rod retina of the skate *Raja*. Cohen himself (cited in Green and Siegel 1975) failed to find any evidence of cone structure, and Dowling and Ripps (1970) have looked exhaustively for conelike structures or physiological activity in *Raja*, with negative results.

Although anomalous and overlapping characters exist (Pedler 1965, Underwood 1968, Cohen 1972, Crescitelli 1972), the differences between rods and cones appear to be great, as can be seen in Table 3. The major identifying features of the two receptor types are the shape of the outer segment relative to the inner segment and the output terminal.

Gilbert (1961), Kato (1962), and Kobayashi (1962) have investigated the elasmobranch retina with conventional histological techniques under the light microscope. Gilbert reported results from 15 species, mostly carcharhinids, while Kato studied two carcharhinid species and Kobayashi investigated *Mustelus* and six Rajaformes. These investigators reported that all retinas were cone-free. However, Kobayashi presented evidence leading to the conclusion that the photoreceptors of *Mustelus*, *Holorhinus*, *Dasyatis*, and *Urolophus* (but not *Narke* or *Raja*) should be physiologically differentiated into rods and cones even though there were no morphological differences. Why these authors were not able to describe the cone photoreceptors present in most of the species studied is not clear. One problem could have been thickness of the paraffin sections. Histological detail in sections thicker than about 5 μm is obscured because one receptor overlies and interferes with the visualization of others. Another possible difficulty is the photolytic tendency of elasmobranch visual cells upon exposure to bright light (Hamasaki et al. 1967).

Table 2. Distribution of duplex retinas in the elasmobranchs: recent studies.

Taxa	Source	Rod-cone ratio/ Remarks
Squaliformes		
Orectolobidae		
Ginglymostoma cirratum	Hamasaki and Gruber (1965)	7-12:1
G. cirratum	Wang (1968)	13:1
Alopiidae		
Alopias vulpinus	Gruber et al. (1975)	5:1
Lamnidae		
Carcharodon carcharias	Gruber et al. (1975)	4:1
Isurus oxyrinchus	Gruber et al. (1975)	10:1
Carcharhinidae		
Carcharhinus falciformis	Gruber et al. (1963, 1975)	11:1
C. longimanus	Gruber et al. (1975)	10:1
C. milberti	Gruber et al. (1975)	13:1
C. springeri	Gruber et al. (1963)	—*
Mustelus canis	Stell and Witkovsky (1973*b*)	100:1
M. canis	Dowling (unpublished observ.)	— electron optics
Negaprion brevirostris	Gruber et al. (1963)	12:1
N. brevirostris	Wang (1968)	12:1
Prionace glauca	Gruber et al. (1975)	8:1
Sphyrnidae		
Sphyrna lewini	Anctil and Ali (1974)	Few cones
S. mokarran	Gruber et al. (1963)	—
Squalidae		
Squalus acanthias	Stell (1972*b*)	50:1 electron optics
Rajaformes		
Rhinobatidae		
Rhinobatos productus	Dunn (1973)	—
Torpedinidae		
Narcine brasiliensis	Ali and Anctil (1974)	12:1
Dasyatidae		
Dasyatis akajei	Tamura et al. (1966)	—
D. americana	Gruber et al. (1963)	—
D. navarrae	Niwa and Tamura (1975)	—
D. sayi	Hamasaki and Gruber (1965)	5:1
Paratrygonidae		
Paratrygon motoro	Ali and Anctil (1974)	7:1

*Dashes indicate data not available.

Table 3. Rod and cone photoreceptors (various sources).

Rod	Cone
1. Outer segment long and slender.	1. Outer segment tapered, conical.
2. Plasma membrane of outer segment encloses disks, continuity of membrane limited to base of outer segment.	2. Plasma membrane continuous with disks along entire length of outer segment, disks open to ventricular space.
3. Periodicity of disk membrane 185–220 Å.	3. Periodicity: 220–225 Å.
4. Disks replaced by basal formation and apical shedding.	4. Disks probably replaced by in situ exchange of materials. New disks not formed.
5. Disks often lobulated.	5. Disks not lobulated.
6. Diameter of inner and outer segments similar.	6. Inner segment larger.
7. Visual pigment contains "rod opsin."	7. "Cone opsin" present.
8. Outer segment connective long, slender, and derived from flagellum by vesicle formation.	8. Connective shorter; derived by formation of flattened microvilli.
9. Oil droplets, polysaccharides absent, mitochondria few.	9. May store oil droplets, polysaccharides in inner segment, mitochondria densely packed.
11. May lengthen in light.	11. May contract in light.
12. Output terminal "rod spherule" with several synaptic units.	12. Output terminal "cone pedicle"; 12–25 synaptic units.
13. Cell destroyed by Iodoacetate.	13. Cell insensitive to Iodoacetate.
14. Index of refraction is 1.41.	14. Index of refraction is 1.38.

Gruber et al. (1963) presented histological evidence at the light micro-scope level that the retina of the lemon shark, *Negaprion*, is provided with cones. Cones were easily differentiated from rods on the basis of size, shape, and staining properties. For example, rods, which predominated in the ratio of 12:1, had a combined outer and inner segment length of 32 μm compared to 19 μm for cones. The inner segment diameters were 2.3 and 4.7 μm, respectively. The staining properties of the paraboloid conferred a character-istic appearance on the inner segments of the cones, making them easily distinguishable from the rods. The cones of *Negaprion* differed from those of other vertebrates in at least one respect: their nuclei were indistinguishable from those of rods. Mention was made of cone receptors in two other carcharhinids, a hammerhead (*Sphyrna mokarran*) and a stingray (*Dasyatis americana*). The cones of *Dasyatis* were described as typically conelike in appearance in contrast to cones of the shark species.

Hamasaki and Gruber (1965) presented further histological evidence of duplex retinas in the nurse shark *Ginglymostoma cirratum* and the stingray *Dasyatis sayi*. Again, morphological differences between the rods and cones of both species were obvious and conformed to the criteria set up by Pedler and Tilly (1964). In receptor ratio and nuclear position the cones of *Gingly-mostoma* were intermediate between those of *Negaprion* and *Dasyatis*. That is, some of the cone nuclei of the nurse shark straddled the outer limiting "membrane," while all of the cone nuclei did so in *Dasyatis*. The rod-cone ratio at the posterior pole of the eye was 7:1 in the nurse shark and 5:1 in the stingray. Counts from different parts of the nurse shark retina estab-lished that there were more cones (ratio 3:2) in the central retina than in the periphery. No evidence of double cones, twin cones, or oil droplets has been reported in any elasmobranch species except for a brief mention by Wolken (1975) of the possible occurrence of oil droplets in 20% of the receptors of *Mustelus*. Yamada and Ishikawa (1965) casually mentioned the existence of a few cones in the retinas of *Dasyatis* and *Mustelus*.

Wang (1968) extended the work on receptor types in the retinas of *Negaprion* and *Ginglymostoma*. He reported a homogeneous distribution of cones in the ratio of 12 rods to 1 cone in *Negaprion*, while in the dorso- and ventromedial retinal fields of *Ginglymostoma*, rods predominated by 7 to 1. The receptor ratio increased to 13:1 in the dorso- and ventrolateral fields. His receptor measurements agreed with those of Hamasaki and Gruber (1965).

In observations involving light- and dark-adapted eyes, Gruber et al. (1963) and Hamasaki and Gruber (1965) did not observe any morphological differences in the photoreceptors dependent upon state of adaptation and thus claimed that photomechanical movements were absent in the elasmo-branchs. Photomechanical movements, present for example in teleosts, are reciprocal contractions and elongations of the rods and cones, often accom-panied by movement of melanin pigment (Ali 1975).

Wang (1968) agreed that the receptors of *Negaprion* and the rods of *Ginglymostoma* were stationary. However, he noted two unusual changes in the cones of the nurse shark with regard to state of adaptation: during light

adaptation (1) the cone inner segment expands and (2) the cone fiber, that portion connecting the cell nucleus to the inner segment, elongates (Figure 9). This was apparently the first description of physiological changes in the cone fiber of any vertebrate during light adaptation. However, it raises the question of where the tissue displaced by the cone pedicles goes. Does the outer plexiform layer become wrinkled? Unfortunately, Wang presents only line drawings of this phenomenon. Photographs would help in clearing up this question.

Tamura and Niwa (1967) reported on the presence of cones in the stingray *Dasyatis akajei*, but the neko shark *Heterodontus japonicus* was said to be cone-free. In a followup investigation, Niwa and Tamura (1975) examined the retinas of nine elasmobranch species, confirming the presence of cones in *Dasyatis* but denying it in *Mustelus*, *Heterodontus*, *Triakis*, *Orectolobus*, *Glyphus* (=*Prionace*), *Etmopterus*, and *Discobatus*. Species differences may account for these observations, but cones definitely occur in *Prionace* (Gruber et al. 1975) and probably in *Mustelus* (Stell and Witkovsky 1973a).

Anctil and Ali (1974) found "a few cones" in the hammerhead *Sphyrna lewini*; they were difficult to identify among the numerous slender rods.

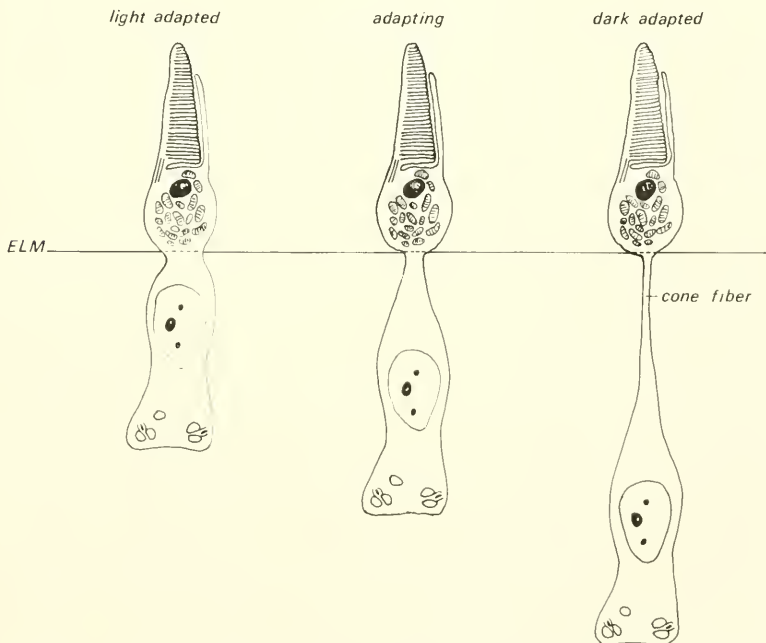*Ginglymostoma Cone Photoreceptor*

Figure 9 Photomechanical movements in *Ginglymostoma cirratum*. While the rods are immobile, the cone fibers apparently elongate during dark adaptation. This arrangement does not change the relative position of rods and cones as occurs during photomechanical movement of teleosts. (Redrawn from Wang (1968); Ph.D. dissertation.)

They also presented clear evidence of cones in the retina of *Narcine brasiliensis* and *Paratrygon motoro*. From a photgraph given in their paper, we estimate the receptor ratio in *P. motoro* at about 8 rods to 1 cone.

To study visual adaptation and its relation to photochemical and neural mechanisms, Dowling and Ripps (1970) searched for an animal that would provide a convenient, long-lasting electrophysiological preparation with a single type of receptor. After careful examination under the light microscope and preliminary study under electron optics, they concluded that retinas of the skates *Raja erinacea* and *R. oscellata* failed to reveal any receptoral detail that could be considered characteristic of cones. This interpretation has been confirmed by A. I. Cohen (personal communication).

Gruber et al. (1975) reported the presence of cones in the retinas of three lamnid and four carcharhinid sharks. Studies of 5-μm-thick paraffin sections under the light microscope revealed that all seven species possess duplex retinas with rods predominating by about 10:1 in the requiem sharks and 6:1 in the more active mackerel sharks. A tally of the recently published photoreceptor literature makes it clear that the prevailing view that the retina of sharks contains but a single type of receptor is in error.

Detailed study of the fine structure of elasmobranch photoreceptors has been reported by Dunn (1973), Stell (1972*a*), and Stell and Witkovsky (1973*a*). The most complete description of receptor morphology, fine structure, and synaptic connection appears to be the work of Stell (1972*b*) on *Squalus*. The receptor layer of *Squalus* forms about 50% of the retina compared to only about 30% in most other vertebrates. Cones make up only 2% of the total receptor population but were easily distinguished from the rods. Figure 10 shows the main ultrastructural difference between the rods and cones at the level of the outer segment.

In *Squalus*, visual pigment containing membranous disks (saccules) of the cone outer segments are open to the ventricular space but are completely enclosed by plasma membrane and probably free floating in rods. In horizontal section the disks are circular in cones but lobulated in rods. These differences imply two very different cellular renewal processes in the rods and cones, as suggested by Dunn (1973). The contiguity of cone disks to the extracellular space appears to be universal in the vertebrates (Cohen 1972), and we have confirmed that the cone outer segments of *Carcharodon* differ from the rods in this unambiguous way.

At the level of the inner segment, the cones bear radiating finlike processes while the rod inner segments appear to be scalloped in outline. This is not a universal feature, however, since Dunn (1973) reported weakly developed fins on the rod inner segments of several vertebrates, including the guitarfish, *Rhinobatos*. Other gross differences characterize the cone inner segments. In *Squalus* they taper from 6 μm at the external "limiting membrane" to 3 μm at the base of the outer segment. Rods are untapering. The mitochondrion-rich ellipsoids of the cones occupy a level below their rod counterpart, which was clearly shown by Stell. As in *Negaprion*, receptor nuclei and cell bodies of *Squalus* can lie at any level in the outer nuclear layer, thus differing, for example, from those of *Ginglymostoma*, in which
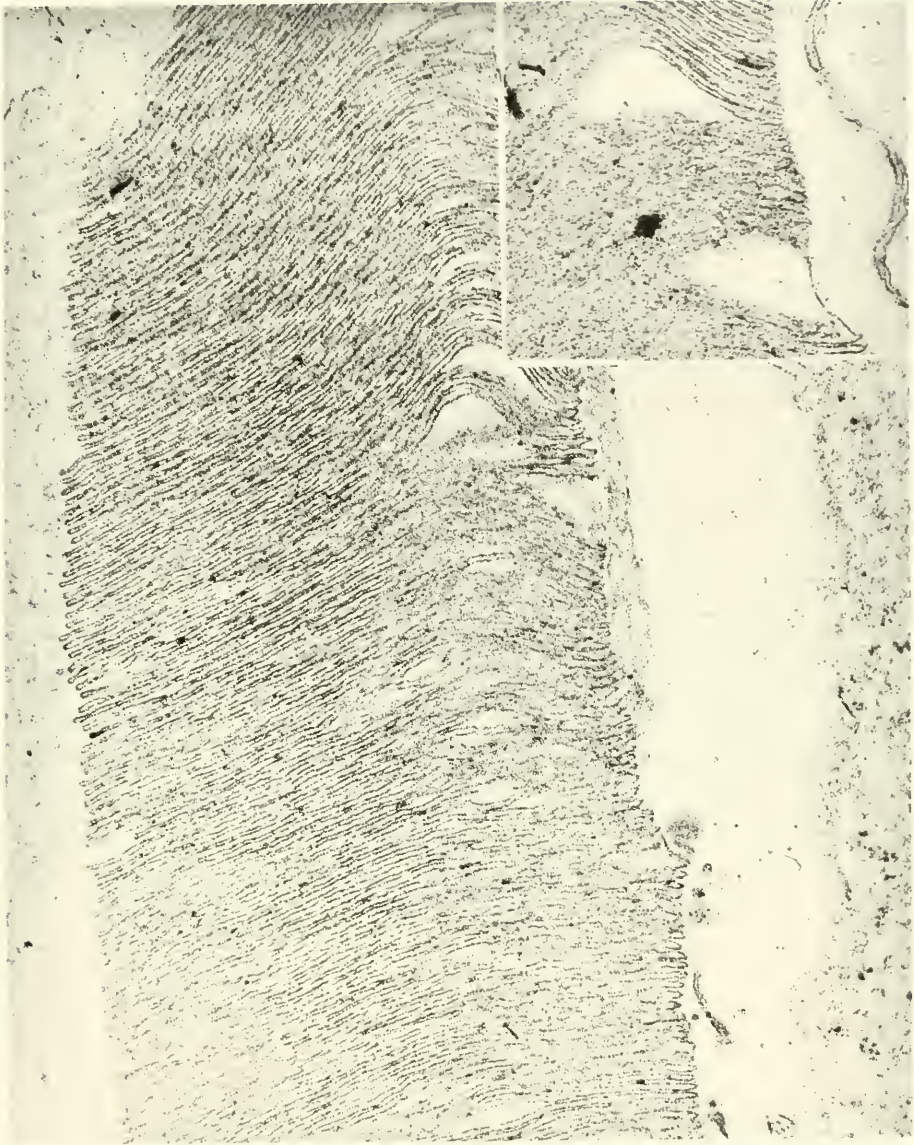
Figure 10 Electron micrograph of a cone outer segment in the retina of *Carcharodon carcharias*. The electron dense layers represent the lipid-rich receptor disks which contain the visual pigment. Infolding of the plasma membrane is clearly shown at the bottom of the inset. It is this infolding which distinguishes the cone outer segment from that of rods. Original magnification 12 000X. Cone disks are approx. 3 μm in width. (From Gruber and Gulley, unpublished observation.)

the cone nuclei straddle the external limiting membrane (ELM) while the rod nuclei of this shark occur well below the ELM.

According to Cohen (1972), the shape and location of photoreceptors suggest that they evolved from ciliated, ependymal-like cells. Ependymal

cells form the lining of the primitive neural tube and ultimately the ventricular spaces of the CNS. This agrees precisely with the location of photoreceptors, which line and project into the remnant of the ventricular space created by the embryological outpocketing of the brain during formation of the eye. The ciliary origin of vertebrate photoreceptors is further suggested by the connective junction between the outer and inner segments. First described by Sjöstrand (1953), the connective has a microstructure similar to that of motile cilia. A pair of ciliary stalks arises from rather typical centrioles located in the inner segment. The cilia, composed of nine pairs of microtubules, are arranged in a circle and connect the inner segment with the outer segment. The cilia continue into the outer segment, running about halfway along its length. Stell observed that the outer and inner segments of *Squalus* are connected not only by a cilium but also by a direct cytoplasmic bridge. He also observed calyceal processes—microvillous structures that extend from the inner segment a short distance over the external part of the outer segments of both rods and cones. Dunn (1973) presented excellent micrographs of the $9 + 0$ ciliary connectives in the photoreceptors of *Urolophus* and *Rhinobatos*.

Photoreceptors terminate with specialized synaptic structures differing considerably in rods and cones. For example, the cone synaptic pedicle is broadly conical, somewhat larger, and contains relatively more invaginating contacts than its rod counterpart. The rod pedicle is roughly spherical and on account of this is often termed the "rod spherule." Stell (1972*b*) described both cone pedicles and rod spherules from the retina of *Squalus*, noting that each contains presynaptic lamellae (synaptic ribbons), as well as synaptic vesicles 400–500 Å in diameter (Figure 11). Typically, vertebrate photoreceptors make four types of contacts: (1) receptor-receptor contacts via direct intercommunication between synaptic pedicles; (2) receptor-receptor contacts via cytoplasmic processes between pedicles; (3) basal surface contacts from dendrites of horizontal and bipolar cells; and perhaps most important, (4) contacts by dendritic processes of horizontal and bipolar cells that invaginate into the synaptic pedicle (Dunn 1973). Stell described in detail contacts within the rod spherules of *Squalus*. Invaginating horizontal cell terminals about 0.25 μm in diameter make at least three specialized types of contacts, along with broad unspecialized apposition. Other invaginating dendrites, most of which are probably unspecialized bipolar neurons, are granule- and vesicle-free and form characteristic "basal junctions," first described by Lasansky (1969). Specialized contacts between bipolar and horizontal cell dendrites were not observed. Because of their relatively low number, Stell's observations on cone pedicles were fragmentary. However, the synaptic structure of cones was rather similar to that found in rod pedicles. Both rod and cone pedicles extend telodendria to the distal layer of horizontal cells. Telodendria were also found within the external plexiform layer. In *Squalus*, the synaptic relations of rods and cones appear similar to those of amphibians, reptiles, and birds rather than of fish and mammals. Stell and Witkovsky (1973*b*) also reported, in the retina of the smooth dogfish *Mustelus canis*, the presence of cones which resembled those of
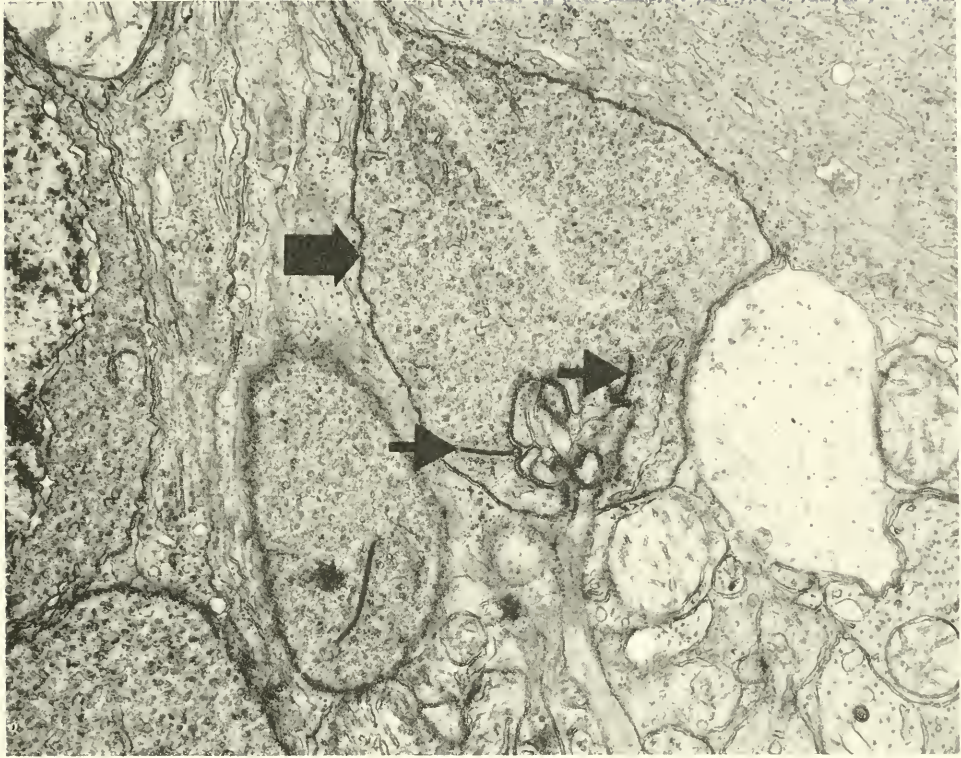
Figure 11 Electron micrograph of a rod photoreceptor terminal in the retina of *Negaprion brevirostris*. Thick arrow points to rod spherule which contains a single synaptic inclusion of invaginating contacts. Thin arrows point to synaptic ribbons. Original magnification 9000X. Rod pedicle is about 8 μm at its widest point. (From unpublished studies by J.L. Cohen.)

Squalus. They estimated the receptor population at no more than a few thousand cones per square millimeter and 100 or more rods per cone.

Horizontal Cells—The layer of neurons proximal (i.e., functionally closer to the brain) to the receptors, the internal nuclear layer, contains the perikarya of horizontal, bipolar, and amacrine cells. Perhaps the most striking feature of the elasmobranch retina is the large cell bodies of the horizontal cells (Kaneko et al. 1976), up to 200 μm across in *Mustelus* (Stell and Witkovsky 1973*b*). The size of the horizontal cells is conducive to penetration with microelectrodes, and thus the electrical activity of these cells has been investigated, for example by Dowling and Ripps (1971*b*), Kaneko (1971), and Naka and Witkovsky (1972). It is only recently, however, that their neural function has been appreciated.

Yamada and Ishikawa (1965) were the first investigators to observe elasmobranch horizontal cells under electron optics. They studied the duplex retinas of *Mustelus* and *Dasyatis* and recognized two layers of horizontal cells: an external layer (closest to the photoreceptors) with large cuboidal

cells and an internal layer of flat squamous cells. These cells were broadly fused laterally, a situation similar to that of cyprinid fish. This differed markedly from the situations in tortoise and man, where horizontal cells make lateral contacts through cytoplasmic extensions. Since the horizontal cells of the elasmobranchs are so broadly coupled (up to 50% of the lateral area, according to Stell), Yamada and Ishikawa suggested that this cell group is functionally a single unit, i.e., an electrical syncitium. Finally, they described conical processes from the external row and long narrow processes from the internal row of horizontal cells, which apparently contact receptor pedicles.

In a preliminary study, Gallego (1972) observed horizontal cells of the nurse shark *Ginglymostoma* under the light microscope by impregnating whole retinas with reduced silver. He had previously postulated (Gallego 1971) that axonless horizontal cells mediate interaction between rods and bipolar cells, while horizontal cells with axons mediate interactions between cones and bipolars. Because sharks were said to possess pure-rod retinas, Gallego felt that the studies of these animals might be crucial to his theory. The layer of horizontal cells in the nurse shark retina stained by reduced silver were indeed axonless, a finding that agreed with Stell's and Kaneko's observations on other elasmobranch horizontal cells. However, Gallego was apparently unaware that Hamasaki and Gruber (1965) and Wang (1968) had reported that cone photoreceptors make up about 12% of the visual cell population in *Ginglymostoma*. Thus his findings on *Ginglymostoma* cannot confirm his suggestion that rods are related to axonless horizontal cells.

Stell and Witkovsky (1973*b*) studied the structure and synaptic connections of Golgi-impregnated horizontal cells of *Mustelus* under the light microscope. They recognized three layers of horizontal cells, all of whose processes reach the external plexiform layer (Figure 12). Cells of the distal layer (H1) are massive, perhaps 200 × 125 × 25 μm, and each probably sends processes into every rod spherule in its field. The flattened horizontal cells of the intermediate layer (H2) form clusters of terminals, and each rod within the field of a cluster may receive a contact. Thus each rod apparently receives contacts from H1 and H2 cells. Cells of the third, most proximal layer (H3), are flattened and stellate in form. They send relatively thick processes vertically and contact cones exclusively. This situation is reversed in teleosts, where H1 cells contact cones and H2 and H3 cells contact rods.

Stell and Witkovsky never observed H3 contacts within rod spherules or H1 and H2 processes associated with cone pedicles. This does not rule out the possibility of rod-cone cross-connections through horizontal cells via photoreceptor telodendria. They noted that elasmobranch horizontal cells with axons have never been observed. Anctil and Ali (1974) confirmed the presence of three rows of horizontal cells in the retina of the hammerhead *Sphyrna lewini*, but did not determine horizontal cell-photoreceptor relationships.

Yamada and Ishikawa (1965) have reported specialized contacts between horizontal cells similar to the so-called gap junction first described by Revel and Karnovsky (1967). Gap junctions between horizontal cells of other elas-
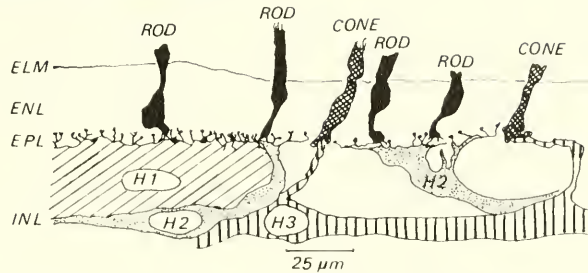
Figure 12   Receptor/horizontal cell organization in the retina of *Mustelus canis*. H1 represents the external layer of horizontal cells which appears to contact rods alone. H2, the intermediate layer, also contacts only rods. The internal layer of horizontal cells (H3) appears to contact cones alone. ELM = external limiting "membrane"; ENL = external nuclear layer; EPL = external plexiform layer; INL = internal nuclear layer. (Redrawn from Stell and Witkovsky (1973*b*). Reproduced by kind permission of the authors and the *Journal of Comparative Neurology* © 1973 Wistar Press.)

mobranchs were also reported by Stell (1966, 1972*a*) and by Hama in a personal communication to Stell (1972*a*). The importance of gap junctions, which may be defined as a special type of close membrane apposition with space between opposing cells reduced to 20–40 Å, is that they form sites of low resistance to electrotonic spread. This implies that horizontal cells are electrically coupled, which Kaneko elegantly demonstrated by recording the S-potential (discussed in detail later) of adjacent horizontal cells. He found that injection of current into one cell polarized neighbors separated by up to five cells. Such electrotonic spread was not found between different horizontal cell layers or between horizontal and bipolar cells. Coupling was confirmed by injection of Procion (fluorescent) dye into horizontal cells which diffused into neighboring cells. Procion did not enter bipolar cells or cells of other horizontal cell layers. Thus evidence was presented favoring Yamada and Ishikawa's (1965) suggestion that horizontal cell layers form an electrical syncitium, which also explained the extraordinarily large receptive fields of S-potentials that originate in horizontal cells.

In a preliminary study Kaneko et al. (1976) investigated completely isolated horizontal cells of several elasmobranchs. The method of isolation involved digesting the retina in trypsin. They produced excellent photomicrographs of individual horizontal cells under Nomarsky optics. Kaneko et al. hope to improve their techniques so that healthy retinal neurons can be isolated and their physiological and biochemical properties characterized.

Bipolar cells—Bipolar cells, as their name suggests, are interneurons that send processes from the internal nuclear layer toward photoreceptors as well as ganglion cells. They thus vertically connect the input and output

stations of the retina, although such direct anatomical cell-to-cell connection may be an oversimplification. Two basic types of vertebrate bipolar cells, each associated with the two photoreceptor types, were described long ago (Schiefferdecker 1886) and their existence was confirmed in sharks (Neumayer 1897).

The only modern work on bipolar neurons of sharks is that of Witkovsky and Stell (1971, 1973). Retinal bipolars of *Mustelus* were examined under the light microscope after Golgi impregnation or vital staining (methylene blue). These were found to conform to the general vertebrate plan: that is, axons were either fine or displayed bulbous terminal expansions (Figure 13). Several other factors were considered in classifying the bipolar cells of *Mustelus*. Thus, five repeatedly observable types were reported. On two of the subtypes there was a clublike process extending into the receptor layer. These processes were first described by Landolt (1871) in the amphibian
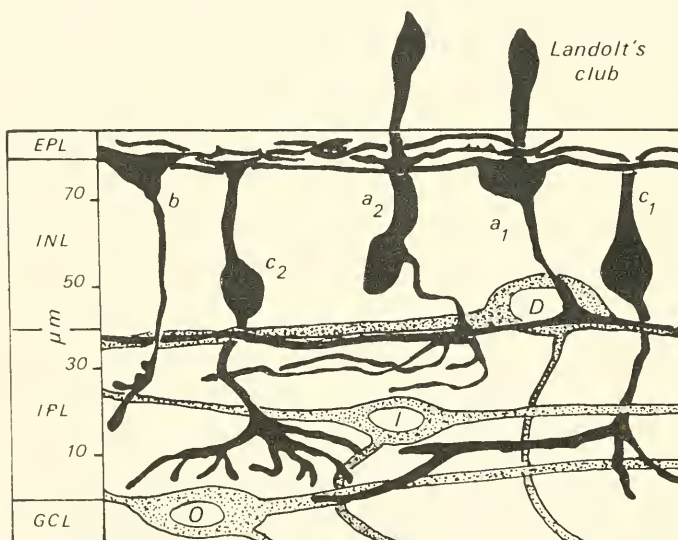

Figure 13 The organization of innerplexiform layer in the retina of *Mustelus canis*. The five classes of bipolar cells and three of the several types of ganglion cells are shown. a_1 = monostratified bipolar cell with cylindrical Landolt's club terminating in distal third of IPL. a_2 = multistratified bipolar with filamentous Landolt's club. b = monostratified bipolar terminating in middle third of IPL; no Landolt's club. c_1 = monostratified bipolar terminating in proximal third of IPL. c_2 = same except multistratified; no Landolt's club. D, I, O, = displaced, intermediate, and ordinary ganglion cells. EPL = external plexiform layer. INL = inner nuclear layer. IPL = internal plexiform layer. GCL = ganglion cell layer. (Redrawn from Witkovsky and Stell (1973). Reproduced by kind permission of the authors and the *Journal of Comparative Neurology* © 1973 Wistar Press.)

retina and are now known as Landolt's clubs. Landolt's clubs (Figure 13) of *Mustelus* vary in external form from filamentous to bulbous. Landolt's clubs in other organisms resemble the photoreceptor inner segment, since both possess ciliary basal bodies and are packed with mitochondria. However, the function of Landolt's clubs is presently unknown (Stell 1972a).

Bipolar cell bodies are arranged in three to four levels in the internal nuclear layer and their principal dendrites extend vertically between horizontal cells and then branch horizontally before contacting the photoreceptor terminals. Bipolar axons course toward the thick (40 μm) internal synaptic layer and there terminate on ganglion and/or amacrine cells in an apparently preferential manner. Thus, the inner synaptic layer is divided into three sublayers that appear to segregate the specific bipolar types with specific ganglion cell types.

Amacrine Cells—Amacrine cells are retinal neurons of the inner nuclear layer found in all vertebrate classes. The characteristic feature, as their name suggests, is that they lack an axon. This is also true of elasmobranch horizontal cells. Little is known of amacrine cells in elasmobranchs. Stell and Witkovsky (1973b) briefly described those of *Mustelus* (Figure 14) as small fusiform cells with incredibly long, bifurcate processes spanning up to 5 mm! Amacrine cells apparently receive input from bipolars and spread information laterally. However, Witkovsky (1971) reported the presence of myelinated fibers of unknown origin entering the retina of *Mustelus* at the optic disk and running to the internal nuclear layer, there synapsing with bipolar and probably amacrine cells. Witkovsky believed these to be centrifugal fibers primarily because of their morphological similarity to efferent fibers in the pigeon retina. The origin of the pigeon efferents was demon-
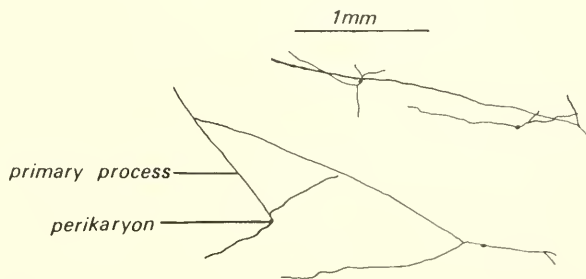

Figure 14 Flat mounted, methylene blue stained amacrine cells of the large variety from the retina of *Mustelus canis*. As their name suggests, these neurons lack an axon but possess exceedingly long processes. If the apparent bilateral symmetry of these cells is real, the horizontal span of amacrines may exceed 5 mm. (Modified from Stell and Witkovsky (1973a). Reproduced by kind permission of the authors and the *Journal of Comparative Neurology* © 1973 Wistar Press.)

strated to be in the isthmo-optic nucleus of the brain (see Cowan 1970, review). Thus it is possible that these fibers represent inhibitory pathways from brain to retina, required to suppress information flow from one eye so that the shark can, for example, attend to signals from the contralateral eye.

Ganglion Cells—The most proximal retinal layer contains the ganglion cells whose axons form the optic tract and thus communicate with the brain. Ganglion cells of elasmobranchs, known since the work of Retzius (1896), have only recently been treated in any detail (Shibkova 1971, Stell and Witkovsky 1973a). Some ultrastructural features of the ganglion cells of *Rhinobatos* have been given by Dunn (1973). As with other retinal neurons, ganglion cells can be divided into several subgroups, typically characterized by size, location, and dendritic arborization. Polyak (1941) recognized no less than six types of ganglion cells in man. Stell and Witkovsky (1973a) distinguished several ganglion cell types but described only those designated as giant ganglion cells (GGC). Shibkova (1971) reported that GGC's make up only a few percent of the neurons of the ganglion cell layers of the retinas of *Squalus* and *Raja*. She estimated the ratio of GGC: medium ganglion cells: small ganglion cells as 1:3:50.

Morphological characteristics of the GGC's of *Mustelus* include large, flattened, stellate perikarya (approximately $10 \times 40 \ \mu m$); nonstratified dendritic arbor with simple radiate patterns; and dendritic spread up to 2 mm. The axons that form the optic nerve arise from axon hillocks, run for a short distance and become completely myelineated, each finally joining several other axons to course as a bundle toward the optic disk. Although the ganglion cell axons of sharks (and teleosts) are myelinated, those of man and other mammals are usually unmyelinated (Sjöstrand and Nilsson 1964); both myelinated and unmyelinated fibers have been reported from the turtle retina (Dunn 1973).

Stell and Witkovsky divided GGC's into three subgroups, depending on the retinal position of the cell body: (1) ordinary GGC's located at the vitreal side of the inner plexiform layer, (2) displaced GGC's located at the scleral side of the inner plexiform layer, and (3) intermediate GGC's found entirely within the inner plexiform layer. The authors present reasonable arguments that GGC's constitute a distinct class of neurons and that the basis for subdivision into ordinary and displaced GGC's is real. Anctil and Ali (1974) confirmed the presence of these three types of GGC's, along with smaller ganglion cells and "glial" cells in the retina of the hammerhead *Sphyrna lewini*.

Dendrites of ordinary GGC's appear to receive input from amacrine and bipolar cells with narrow horizontal spread, while the larger GGC's receive contacts from neurons with greater horizontal spreads. To distinguish patterns of ganglion cell organization and to characterize the dendritic arbor, Stell and Witkovsky counted the number of dendritic branches (Figure 15) at various distances from the perikarya of a number of displaced and ordinary GGC's. They also diagrammed the extent and form of dendritic fields as a function of retinal location. Results of these studies indicated that the
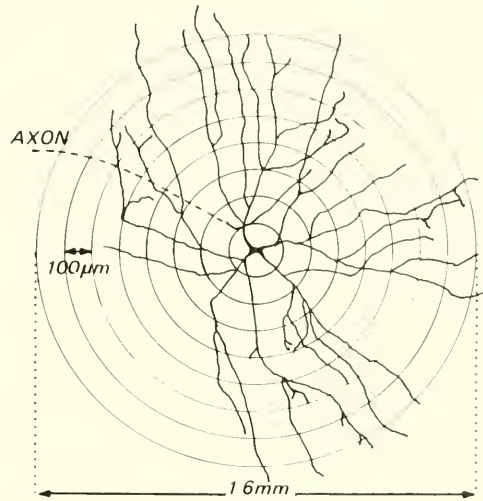
Figure 15 Flat mounted, methylene blue stained giant ganglion cell from the retina of *Mustelus canis.* Concentric circles were used by Stell and Witkovsky to map and quantify the organization of the dendritic arbors of giant ganglion cells. (Redrawn from Stell and Witkovsky (1973*a*). Reproduced by kind permission of the authors and the *Journal of Comparative Neurology* © 1973 Wistar Press.)

average dendritic field diameter is very nearly equal to the average distance between displaced GGC's, and thus this cell group forms a gridwork of overlapping fields. They concluded that each retinal area of overlap is provided with dendrites of seven to eight cells. While different dendritic patterns between different classes of GGC's were detected, dendritic density in all cases fell exponentially from the center of the cell to a value of 1% at the periphery.

For every ganglion cell counted, six times as many small (9 × 12 μm) ellipsoidal cells were observed in the ganglion cell layer. Stell and Witkovsky suggested that these are glial cells—perhaps sheath cells associated with the myelinated optic nerve axons. Still, they could be ganglion cells with unmyelinated fibers. One piece of evidence against this view is that in the *area centralis* of *Mustelus* the density of ganglion cells increases dramatically while the number of "glial" cells remains the same. Franz (1931) estimated the increase at from 800 to 2500 cells/mm^2.

Shibkova (1971) reported on cytoarchitecture and histochemistry of retinal ganglion cells in *Squalus* and *Raja.* Many of her findings were confirmed in Stell and Witkovsky's (1973*b*) independent study on *Mustelus.* In addition she found that the glycogen-filled small and medium ganglion cells are histochemically less active than GGC's; this suggested that the main

visual elements are the GGC's, which presumably feed information to the primary visual areas of the brain. The function of the small cells was "less clear."

Finally, Walls (1942) mentioned that displaced retinal neurons such as displaced GGC's are indicative of a crude retina. However, Dunn (1973) reported that displaced ganglion cells are known from all vertebrate classes with the possible exception of teleosts, and therefore the significance of these displaced neurons is not known.

Visual Pigments—Visual pigments are organic dyes composed of a protein, opsin (molecular weight about 27,000), complexed onto a short chain prosthetic or chromophore molecule related to Vitamin A. These pigments are located in the photoreceptors; specifically, rhodopsin constitutes up to 35% dry weight of the rod outer segments (Kropf 1972). While most retinal components are relatively transparent, visual pigments absorb light and give the retina its characteristic purple or pink color. The early retinologists were almost certainly aware of the coloration associated with the retina (Crescitelli 1972), but it was Boll (1876) who first reported that the color quickly fades in light. He correctly associated this bleaching with rod photoreceptors and related it to the mechanism of vision. He also observed *sehrot* in the retina of sharks, and Krause (1889) reported *sehpurpur* from the rod outer segments of a ray. However, the most important pioneer work in visual pigments was that of Kühne (Crescitelli 1972), who coined the term "rhodopsin," extracted and characterized this material, produced a crude absorption spectrum, and observed regeneration of rhodopsin from its products of bleaching.

It was not until 1936 that Bayliss et al. first investigated elasmobranch visual pigments in any detail. Since then retinas from about a dozen species have been extracted, primarily in digitonin, and their visual pigments characterized (Table 4). In addition, Denton and Nicol (1964) and Dowling and Ripps (1970) have investigated elasmobranch visual pigments in situ by the methods of differential density and fundus reflectometry.

The absorbance maximum of the ordinary elasmobranch visual pigment lies at about 500 nm, which is typical of rhodopsin. The chromophore has been characterized as Vitamin A_1–based retinene (i.e., Beatty 1969).

Bayliss et al. (1936) and Clarke (1936) independently suggested that fishes inhabiting the deeper ocean might be visually adapted to the spectral quality of light at depth. This was confirmed by Denton and Warren (1956), who reported that the retinas of three teleosts living below 500 m contained a golden colored pigment (chrysopsin) in high density. These fishes had visual pigments shifted some 20 nm toward the blue and were thus well adapted to make use of the fraction of daylight that reaches that depth. In a remarkable example of parallel evolution, Denton and Shaw (1963) reported that retinas of three elasmobranch species caught at 1150 m contained a golden pigment similar to the chrysopsin of deep-sea teleosts. While the chromophore of elasmobranch chrysopsin was not chemically identified and the visual pigments were not checked for homogeneity, the authors clearly demonstrated that these pigments were environmentally tuned. Of the three shark retinas

Table 4. Visual pigments of the elasmobranchs.

Taxa	Peak of the absorption spectrum (λ_{max} in nm)	Source	Remarks
Squaliformes			
Carcharhinidae			
Galeorhinus laevis (prob. *M. canis*)	—*	Wald (1939)	Chromophore identified as retinene based on Vitamin A_1
Mustelus californicus	497	Crescitelli (1972)	Pigment tested for homogeneity, chromophore identified as retinene
Negaprion brevirostris	501	Bridges (1965a)	Pigment tested for homogeneity, chromophore identified as retinene
Scyliorhinidae			
Scyliorhinus canicula	505	Bayliss et al. (1936)	—
S. canicula	500	Denton and Nicol (1964)	In situ determination
Squalidae			
Centrophorus squamosis	482	Denton and Shaw (1963)	Blue shifted "chrysopsin" discovered in deep-sea sharks
C. squamosis	484	Denton and Nicol (1964)	In situ
Centroscymnis coeliolepis	472	Denton and Shaw (1963)	—
C. coeliolepis	472	Denton and Nicol (1964)	In situ
Deania calcea	484	Denton and Shaw (1963)	In situ
D. calcea	484	Denton and Nicol (1964)	In situ
Squalus acanthias	500	Wald (1939)	—
S. acanthias	500	Denton and Nicol (1964)	In situ
S. suckleyi (=*acanthias*)	497.5	Beatty (1969)	Method of partial bleaching, chromophore identified as retinene
Rajaformes			
Rhinobatidae			
Rhinobatos productus	497	Crescitelli (1972)	—
Rajidae			
Raja clavata	510	Bayliss et al. (1936)	—
R. erinacea	500 ⎫	Dowling and Ripps (1970)	Fundus reflectometry
R. oscellata	500 ⎭		
R. binoculata	497	Beatty (1969)	—
Paratrygonidae			
Paratrygon motoro	499	Muntz et al. (1973)	Product band upon bleaching characteristic of retinal oxime
Myliobatidae			
Myliobatus californica	500 ± 2	Munz (1965)	—
Rhinoptera steindachneri (?)	500 ± 2	Munz (1965)	—

*No data.

examined, the visual pigment of *Centroscymnus* was most blue shifted (472 nm) while *Centrophorus* was intermediate at 482 and *Deania* was least shifted (484 nm). Using an entirely separate technique, Denton and Nicol (1964) confirmed the λ_{max} position at 472 and 484 nm for *Centroscymnus* and *Deania*, respectively. Parallel visual pigment adaptations have recently been reported from invertebrates and marine mammals (Lythgoe 1972).

In a noteworthy study Pepperberg et al. (1976) investigated the effects of placing various isomers of retinal (the chromophore of rhodopsin) on a physiologically active but strongly bleached retina. Using the all-rod retina of *Raja*, they first intensively light–adapted the retina with green light, which bleached about 90% of the rhodopsin. Since the retina was isolated from the pigment epithelium, further visual pigment regeneration could not take place. Thus, the amplitude of the receptor potential reached a stable plateau determined mainly by the amount of rhodopsin available in the rod outer segments. Next, aliquots of the *all-trans* isomer of retinal were dropped on the retina. As expected, no change in sensitivity was recorded since the normal effect of light on unbleached visual pigment is to isomerize the chromophore to the *all-trans* form. When the *11-cis* isomer of retinal was sprayed on the retina a dramatic increase in sensitivity was recorded. Most unexpected was the increase in sensitivity when *9-cis* retinal was placed on the retina. Densitometric evidence presented led to the conclusion that external application of *11-cis* retinal rapidly promotes the formation of rhodopsin; application of *9-cis* retinal similarly forms isorhodopsin, a visual pigment not naturally found in skate photoreceptors. Thus, the receptor mechanism that subserves those sensitivity changes dependent upon concentration of visual pigment is able to use both rhodopsin and isorhodopsin. This was apparently the first demonstration of changes in receptor sensitivity directly dependent upon *11-cis* retinal.

It is assumed that rhodopsin occurs only in the outer segments of rod visual cells. Cone pigments of mixed retinas are not ordinarily obtained through the extraction procedures. Recently, however, analysis of the light-absorbing properties of individual cone outer segments of the goldfish has been achieved by microspectrophotometry (Marks 1965). Harosi and Gruber attempted to characterize the cone outer segments of *Negaprion* by this method but were unsuccessful. Thus, nothing is known of the cone pigments of elasmobranchs.

Retinal Electrophysiology—Electrical recording from the fish retina was first attempted by Dewar and McKendrick (1873). However, it was not until the early 1960's that researchers turned toward elasmobranchs, to use their retinas for electrical recordings to answer questions of ecology, behavior, physiology, and pharmacology as they pertain to the animal in particular and visual science in general.

Electroretinogram (ERG)—The early electrophysiological studies on elasmobranchs concentrated on recording a massed response of several retinal cell types known as the ERG to determine waveform and other

properties and to correlate the spectral sensitivity of the animal with its environment.

The electroretinogram can be observed by recording the electrical potential difference between a wick electrode placed on the surface of the cornea and an indifferent electrode situated either behind the eye or subcutaneously in the head. Alternatively, a fine chlorided silver wire may be placed in the vitreous humor through a puncture in the sclera. The ERG may also be recorded from what is termed an "eyecup preparation." With this method the eye is removed from the animal and the anterior section, including cornea, lens, and humors, is removed. The eye is then placed in a chamber through which oxygen or another gas mixture flows, to keep the retina alive. A wick electrode is used for recording.

In general the ERG consists of a series of negative and positive waves (Figure 16). The a-wave or PIII is a negative deflection whose distal or leading edge is thought to arise from the photoreceptors. Recent evidence shows that the proximal part of the ERG originates from glia (Witkovsky et al. 1975). The positive wave following the a-wave is termed the b-wave (PII) which apparently originates in the Müller fibers (Miller and Dowling 1970). A slow positive wave termed the c-wave (PI) follows and this originates in the pigment epithelium (Steinberg et al. 1970). A positive off effect, termed the d-wave, is sometimes seen.

Kobayashi (1962) recorded two types of ERG's in the eyecup preparation (i.e., cornea, lens, and vitreous removed) of the dogfish *Mustelus manazo*: a fast diphasic and a slow, negative monophasic form. He noted that the fast type tended to be recorded from the ventral region, while the slow form apparently originated from the dorsal side of the retina. After partial dark adaptation, weak light stimuli of short duration evoked a positive monophasic wave. In the completely dark-adapted eye, the ERG was followed by a slow negative wave. Long-duration stimuli did not evoke an off response. With more intense light, a fast negative potential preceded the positive one. The slow response was a negative deflection that increased in amplitude: an off
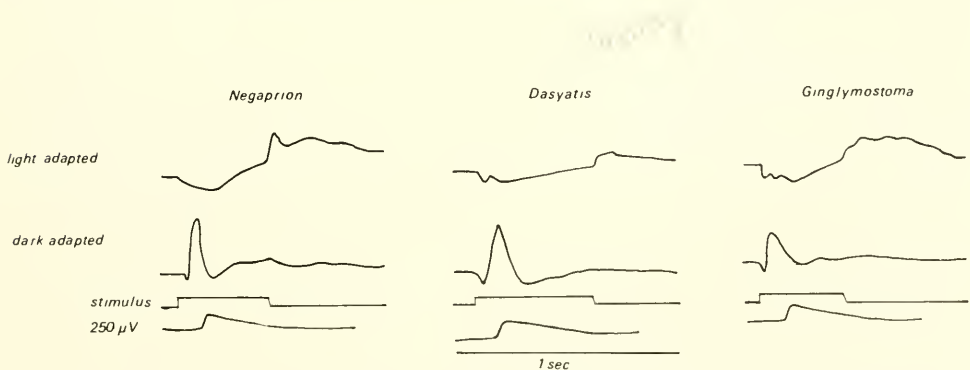

Figure 16 Comparison of light- and dark-adapted ERG waveforms in three elasmobranch species. Calibration 2.5 s; 250 µV. (Taken from Hamasaki and Bridges 1965 with kind permission of the authors and *Vision Research* © 1965 Pergamon Press Limited.)

effect was never observed. Kobayashi noted that thresholds for the slow response were 10 times higher than those for the fast type.

In spite of finding the maximum sensitivity at 500 nm for both the light- and dark-adapted preparations, Kobayashi nevertheless considered that the two different responses were subserved by different mechanisms: the slow type from a photopic mechanism, while the fast type derived from a scotopic mechanism. However, this seems reversed, since intracellular record- ings from the photoreceptors showed that the cone responses exhibited a faster time course of decay than did the rods (Baylor and Fuortes 1970).

In a similar series of experiments, again using the eyecup preparation, Kobayashi (1962) investigated properties of the ERG in a number of rays and skates, including *Narke, Holorhinus, Dasyatis, Urolophus, Platyrhina,* and *Raja*. Results were similar to those of the dogfish.

Although Kobayashi was the first to use elasmobranchs for ERG studies, many of the responses he recorded were negative deflections and those that were positive were small in amplitude and changed the sensitivity by only 2–3 log units during dark adaptation. Granit (1947) has shown that such responses are typical of anoxic and deteriorating preparations.

To overcome these difficulties, Hamasaki and Bridges (1965) and Hamasaki et al. (1967) studied the ERG in the intact preparation of the lemon shark *Negaprion*, the nurse shark *Ginglymostoma*, and the stingray *Dasyatis sayi*.

By inserting a fine chlorided wire into the vitreous of the eye of an immobilized animal, they recorded ERG's with positive components whose sensitivities increased by at least 6 log units (i.e., 1 million-fold) during dark adaptation, thus demonstrating that their preparations were in excellent con- dition.

During these experiments it was found that in the light-adapted state the predominant form of the ERG was negative; it changed to a positive response during dark adaptation. Large, positive off effects were seen in the light-adapted state, but little or no off effect was recorded during a dark adaptation (Figure 16). No c-wave was seen in any of the elasmobranch ERG's studied by Hamasaki and Bridges (1965).

O'Gower and Mathewson (1967) also studied the ERG of the lemon shark, noting only an increase in the amplitude of the ERG when stimulus intensity was changed. The authors did not report a change in latent period or waveform of the ERG when stimulus wavelength was changed.

In addition to the usual waveform, Hamasaki and Bridges (1965) observed a series of rhythmic potentials that appeared after 1 min of dark adaptation but disappeared as dark adaptation continued. High-intensity stimuli blocked these oscillations.

In addition, depending upon intensity of the first flash, an ERG ordinarily evoked by a second flash of light might be completely suppressed. At stim- ulus levels 3 log units above threshold, the response amplitude to a second flash is reduced. With full-intensity stimuli, the first flash completely sup- pressed any ERG response to a second flash. Duration of the first flash was also an important factor in the amount of suppression produced. As the

duration of the first flash was decreased from 1500 ms, the size of the response to the second flash increased, i.e., suppression was reduced. State of adaptation also influenced suppression. With a light-adapted animal suppression could not be evoked until after 5–10 min in the dark. Complete suppression did not occur until after 40 min of dark adaptation. As the interval between the two flashes was increased, the suppression was diminished. However, decreasing the interval to less than 5 s increased the suppression. Hamasaki and Bridges confirmed the presence of suppression in the retinas of *Negaprion*, *Ginglymostoma*, and *Dasyatis*. Similar findings were made by Dowling and Ripps (1970) for the skate *Raja*.

To determine whether the suppression is a local retinal event, Hamasaki and Bridges made simultaneous ERG and tectal recordings. Even though the ERG was completely suppressed with a second flash, a near-normal discharge was detected from the tectum, indicating that signals were being transmitted from the retina to the optic tectum. This conflicts with the results of Dowling and Ripps (1970), who showed a close correspondence of b-wave and ganglion cell sensitivity in the skate. Since the ganglion cells represent the final output of the retina, it follows that tectal responses should reflect any suppression in the ERG after a second flash. Dowling and Ripps suggested that since the ERG is a massed response, enough of the retina might remain responsive enough to direct or stray light to evoke a response in the tectum.

Receptor Potentials—Direct recordings from elasmobranch photoreceptors have not yet been reported. However, the receptor potential of the skate *Raja* has been isolated by application of sodium aspartate to the eyecup preparation (Dowling and Ripps 1971a, 1972). This technique was first used to evoke receptor potentials in the toad retina (Furukawa and Hanawa 1955).

After immersion of the eyecup in Ringer's solution containing aspartate, the b-wave of the ERG is lost, leaving the a- and c-waves (Figure 17). Visualization of the a-wave is increased in the presence of aspartate, due to the absence of the b-wave. With removal of the pigment epithelium, from which the c-wave originates (Steinberg et al. 1970), only the a-wave remains. Current evidence points to the distal edge of the negative a-wave as originating from the photoreceptors (Sillman et al. 1969), while the proximal part is probably derived from glial cells (Witkovsky et al. 1975).

Cohen et al. (1977) used the receptor potential of *Negaprion* to measure the spectral sensitivity of its retina.

Horizontal Cell Responses—Horizontal cells are characterized by having all of their processes end in or about the outer plexiform layer, where they contact the photoreceptors (Rodieck 1973). In fishes, including elasmobranchs, they are very large. It is because of their size that they have been much studied by the method of intracellular recording. Yet, in spite of this wealth of information, the exact functional role of horizontal cells is not known.
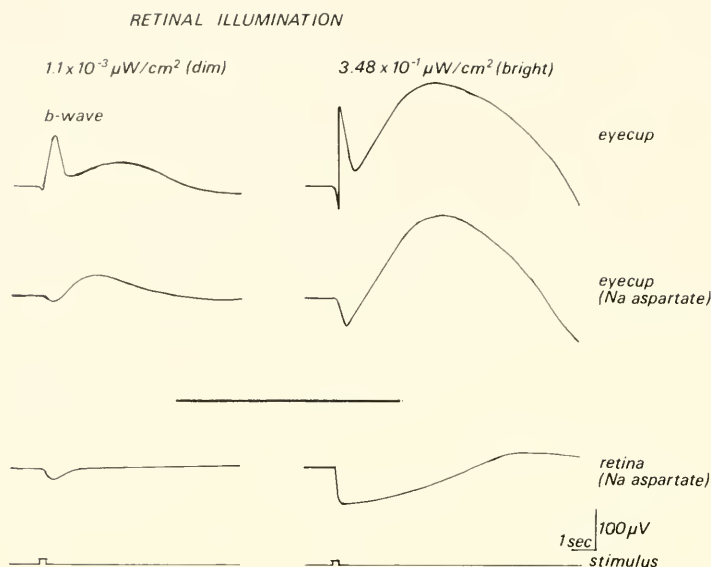
RETINAL ILLUMINATION

Figure 17   Effect of sodium aspartate on the massed response of the retina of *Raja*. Responses evoked by bright and dim 0.2 s flashes of light are shown. Immersion of the preparation for 3 min in Na aspartate abolished the b-wave of the ERG. Removal of the retinal epithelium eliminated the c-wave. Thus the aspartate-treated retina produces a massed response consisting primarily of the a-wave, most of which represents the activity of the photoreceptors. (Modified from Dowling and Ripps 1972. Reproduced by kind permission of the authors and the *Journal of General Physiology* © 1972 Rockefeller University Press.)

Horizontal cells respond to light with a graded, hyperpolarizing potential (Figure 18). This response, termed the "S-potential," is divided into two types. The first, referred to as the L- or luminosity type, responds to all wavelengths of light with hyperpolarizing potentials. Because of this it is thought to encode luminosity or brightness information. The second type of S-potential is termed the C- or chromaticity type. Cells of the chromaticity type respond with hyper- and/or depolarizing potentials, depending on stimulus wavelength. New evidence has shown that in the turtle and goldfish these responses are the result of feedback from receptors via horizontal cells onto other receptors (Fuortes and Simon 1974, Stell et al. 1975*b*).

Most S-potentials recorded from elasmobranchs are of the L-type (Tamura et al. 1966, Tamura and Niwa 1967, Dowling and Ripps 1971*b*, and Niwa and Tamura 1975). However, C-type potentials were recorded from the retina of *Dasyatis akajei* (Tamura et al. 1966, Tamura and Niwa 1967, and Niwa and Tamura 1975), which possesses both rods and cones (Table 5).

Properties of elasmobranch S-potentials are similar to those recorded from other animals. However, some differences exist; these will be dealt with later.
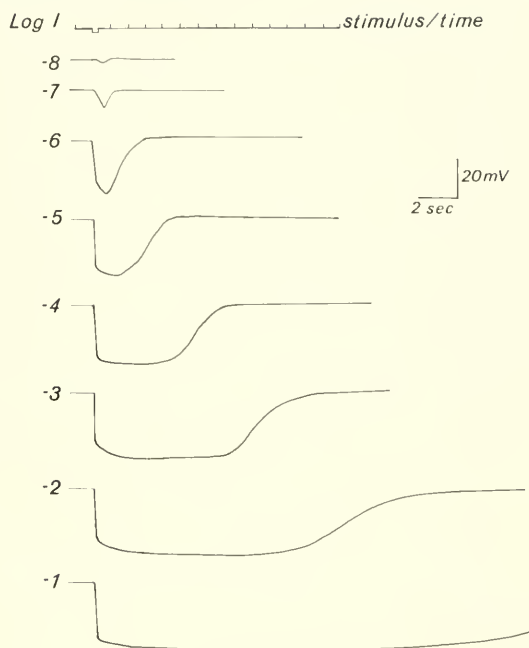
Figure 18 Relation between stimulus intensity and horizontal cell activity in the dark-adapted retina of *Raja*. Maximum stimulus intensity was 1.22 mW/cm^2; duration was 0.2 s. Both amplitude and duration of S-potential are directly proportional to log stimulus intensity. (Taken from Dowling and Ripps 1971b. Reproduced by kind permission of the authors and the *Journal of General Physiology* © 1971 Rockefeller University Press.)

As the area of a photic stimulus is increased, the response amplitude of the S-potential also increases (Norton et al. 1968). This area effect, known from many vertebrate eyes, has also been confirmed by Dowling and Ripps (1971b) for the skate.

The retinal area over which light will evoke a response from a given cell is termed the "receptive field" of that cell. Dowling and Ripps (1971b) have measured receptive fields of skate horizontal cells using bright stimuli. The average diameter was 3 mm. Upon reduction of stimulus intensity, receptive fields increased to 4 mm. Receptive fields up to 10 mm were reported from the horizontal cells of *Mustelus* by Kaneko (1973).

The large size of these receptive fields can probably be attributed to electrical connections between horizontal cells of the same layer. Yamada and Ishikawa (1965) provided anatomical evidence for the existence of gap junctions between adjacent horizontal cells; Kaneko (1973) provided the electrophysiological evidence for electrical coupling by showing that current passed into a horizontal cell can be recorded in an adjacent horizontal cell.

Table 5. S-potentials in elasmobranchs.

Animal	Peak sensitivity (nm)	Response type	Reference
Heterodontus japonicus	494	L	Tamura et al. (1966) Tamura and Niwa (1967)
Mustelus manazo	494	L	Niwa and Tamura (1975)
Triakis scyllia	494−525	L	Niwa and Tamura (1975)
Orectolobus japonicus	494	L	Niwa and Tamura (1975)
Dasyatis akajei	525, 494, 584	L C	Tamura et al. (1966) Tamura and Niwa (1967) Niwa and Tamura (1975)
Raja oscellata	500	L	Dowling and Ripps (1971*b*)

Bipolar Cell Potentials—Bipolar cells are neurons that transmit signals from the photoreceptor cells vertically to the amacrine and ganglion cells. Kaneko (1971) was the first to successfully record from elasmobranch bipolar cells. Using the dogfish *Mustelus*, he found that bipolar cells exhibited resting potentials of −30 to −40 mV. Photic stimuli evoked a two-phase depolarizing response: the first, a transient depolarization, was followed by a smaller phase of maintained depolarization. Hyperpolarizing responses such as those recorded in other vertebrates (Werblin and Dowling 1969) were not reported by Kaneko.

Similar results were given by Ashmore and Falk (1976) from the bipolars of *Raja* and *Scyliorhinus*. Resting potentials of −45 to −60 mV were found with a maximum response to light of 20 mV from the resting potential, i.e., −65 to −85 mV. Responses to centered spots of light consisted of a transient depolarization followed by a sustained depolarization maintained for the duration of stimulation (Figure 19). Again, hyperpolarizing responses were not found.

Receptive field centers in the dark-adapted preparation were on the order of 150 μm with only a barely detectable antagonistic effect in response to illumination in the periphery.

To our knowledge, there have been no recordings from elasmobranch amacrine cells.
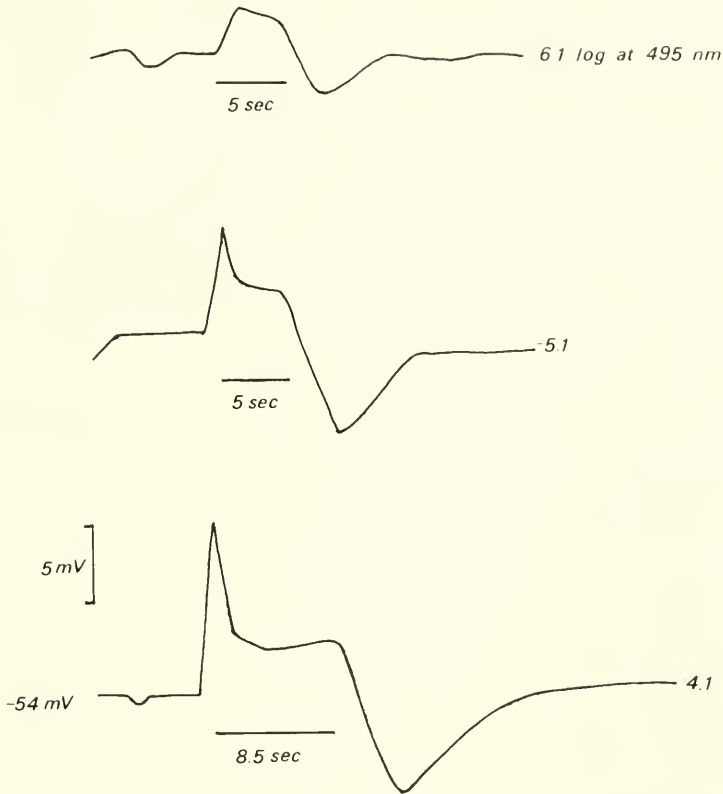
6 1 log at 495 nm

5 sec

-5.1

5 sec

5 mV

-54 mV

4.1

8.5 sec

Figure 19   Bipolar cell responses from the retina of *Scyliorhi-
nus canicula*. Cell responded to long duration (horizontal bars)
flashes of 495-nm light. Numbers to the right represent the
value of the neutral density filter attenuating the 540-nm
diameter circle of light. Electrode penetrated 80 μm from the
vitreal surface. (Taken from Ashmore and Falk 1976 by kind
permission of the authors and the *Journal of Physiology*
(London) © 1976 Cambridge University Press Limited.)

Ganglion Cell Responses—Ganglion cells are tertiary neurons whose
axons form the optic nerve. Their responses represent the final output of the
retina.

Extracellular recordings show that ganglion cells respond to an increase or
decrease in light stimuli with all-or-none nerve action potentials. The recep-
tive field of ganglion cells is organized into a center region and a concentric
surround whose responses are of opposite sign and are thus antagonistic.
Responses obtained by recording in the center of a receptive field differ
depending on the type of cell from which the response is being recorded. A
small spot of light in the receptive field center that evokes a burst of spikes
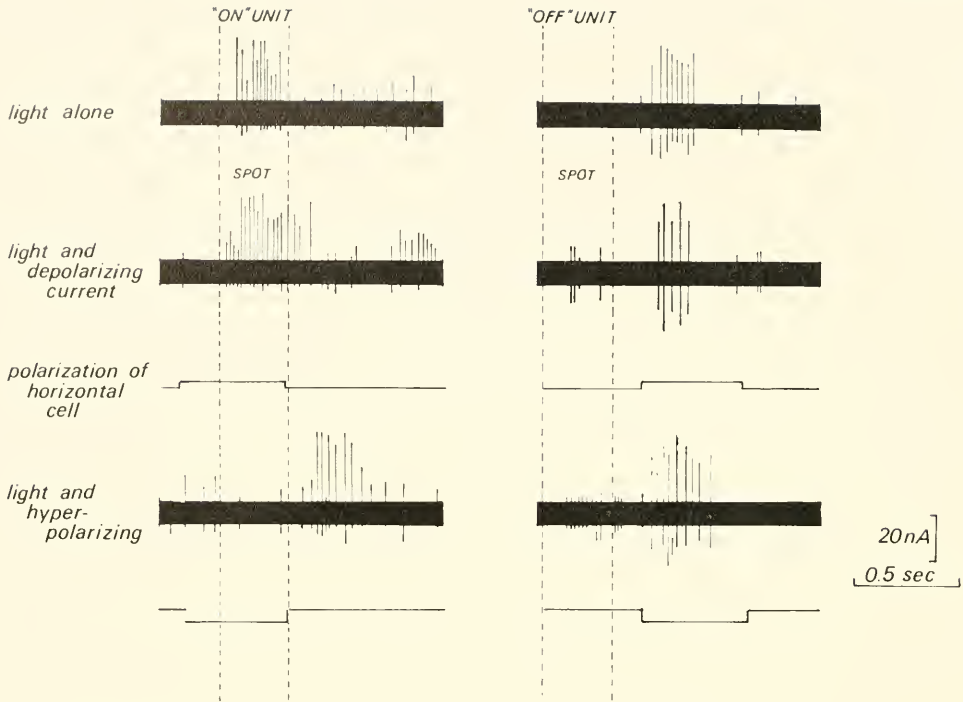characterizes the "on-center" cell (Figure 20). This same stimulus applied to

Figure 20 Responses of ganglion cells in the retina of *Mustelus canis*. Scheme shows the interaction between light and injection of current into a horizontal cell connected through the retina to a ganglion cell. Response of "on" units to light is enhanced by subliminal depolarization of an interconnected horizontal cell. Hyperpolarization results in depression of the ganglion response to light. The inverse is observed with "off" units. (Taken from Naka and Witkovsky 1972 by permission of the authors and the *Journal of Physiology* (London) © 1972 Cambridge University Press Limited.)

the surround will inhibit spontaneous firing when turned on but will evoke firing of the cell when extinguished. A second type of ganglion cell, the "off-center" type, also occurs. Onset of illumination in the center of this cell causes inhibition, while offset causes excitation. In the surround the opposite effect is seen. A third type of cell is termed the "on-off" type. All three types have been found in elasmobranchs. Stell et al. (1975) reported that most of the units in the dogfish *Mustelus* were of the on-center variety, while Dowling and Ripps (1970) reported finding an equal number of on-center and off-center units in the retina of *Raja*.

In addition to the units just described, Stell et al. (1971) found ganglion cells in *Mustelus* whose receptive center responded at offset as well as at onset of illumination. When the stimulus was moved to the periphery, the cell responded both at on and at off. These cells were termed "double opponent cells." However, the conditions under which they were obtained were not specified. Double opponent receptive fields were described for the

goldfish by Wagner et al. (1960) and by Daw (1968) but these refer to an organization based on chromatic stimuli. With the low number of cones found in the retina of *Mustelus* (Stell and Witkovsky 1973*b*), the existence of such a chromatic coding system seems doubtful. Thus, from the description of Stell et al., the responses seem typical of on-off cells.

Large receptive field sizes seem to predominate in the elasmobranchs. Dowling and Ripps (1970) reported a center diameter of 1.5 mm, while the surround comprised an annular region extending at least 3 mm from the border of the center. In the dogfish *Mustelus*, Stell et al. (1975) reported ganglion cell receptive field sizes of 1–3 mm. They suggested that the receptive field centers are determined by spread of the ganglion cell dendrites, since the diameters of the receptive field centers approximated dendritic field diameters measured by Stell and Witkovsky (1973*a*).

Barlow et al. (1957) found that the level of light adaptation influenced receptive field size in the cat retina. Similar results were reported by Stell et al. (1975), who showed that ganglion cell receptive fields shrink during dark adaptation.

Although it is known that the receptive fields of vertebrates, including elasmobranchs, are organized into an antagonistic center surround system, exactly what part the various distal neurons play in producing this system is unknown. To help clarify the role played by the horizontal cells in the organization of the receptive field, Naka and Witkovsky (1972) undertook a series of studies using the dogfish *Mustelus*.

By injecting current directly into dogfish horizontal cells and recording from the ganglion cells, they classified the responses into two types, A and B. Type A cells gave an on response to depolarizing current, an on-off response to hyperpolarizing current, and an on response to a spot of light in the center of the receptive field. Type B ganglion cells responded in the opposite manner; that is, they gave an off response to a depolarizing current, an on response to hyperpolarizing current, and an on response to an annulus of light. Polarization of a horizontal cell evoked responses from both type A and B ganglion cells, thus showing that these cells are not driven by any particular horizontal cells.

It was also found that a subthreshold depolarizing current, which cannot evoke a response by itself, enhances an on discharge evoked by a light stimulus. Hyperpolarizing current caused a depression of the on responses to light stimuli.

Since all electrically stimulated horizontal cells evoked responses in ganglion cells, it was concluded that all ganglion cells in the dogfish retina receive signals from the horizontal cells. These signals are mediated by bipolar cells and probably reflect the activity of bipolar cell membranes (Naka and Witkovsky 1972).

Flicker—The ability to resolve intermittent stimuli has been shown to be a property of the photoreceptors (Rodieck 1973). When critical frequency of flicker fusion (cff) is measured in animals with duplex retinas the resultant cff-vs-intensity curve always shows a "kink," marking a changeover

from rod to cone vision. It is thought that rods follow only slow temporal changes while cones resolve higher flash rates (Graham 1965). This has recently been confirmed by intracellular recordings from turtles, which showed that rods had a slower time course than cones (Baylor and Fuortes 1970).

It is not surprising, then, to find that elasmobranchs with very few cones can resolve intermittent stimuli only at very slow rates. Kobayashi (1962), monitoring the ERG in *Mustelus*, reported a cff of 10 Hz and 6 Hz in *Narke* and *Urolophus*, respectively, while Dowling and Ripps (1970) found a cff of 5 Hz for the skate *Raja*. In contrast to these is the ability of the lemon shark *Negaprion brevirostris* to follow intermittent stimuli at a higher rate, up to 45 Hz (O'Gower and Mathewson 1967; Gruber 1969, 1975; Gruber and Hamasaki, in preparation), which is reasonable in light of their duplex retina (Gruber et al. 1963).

Although a discontinuity in the curve relating cff to intensity and the ability to resolve intermittent stimuli at high rates is indicative of a duplex retina, recent work on the skate retina has shown that this might not necessarily be so. Green and Seigel (1973, 1975) demonstrated that under certain conditions responses from the all-rod retina of the skate produced a double-branched cff log I curve. Stimuli up to 30 Hz were resolved. This was much higher than the value given by Dowling and Ripps (1970). Green and Seigel came to the unorthodox conclusion that both parts of the curve rose as a result of a single photoreceptor having only one photopigment. These were presumably rods, since the spectral senstivity of both high- and low-intensity segments of the cff log I curve followed the action spectrum of rhodopsin. The high cff was obtained by measuring not only the b-wave of the ERG but also the receptor potential and S-potential. The exact mechanism was not described, but by recording the S-potential it was shown that to follow high-frequency stimuli requires that certain conditions be met: (1) the stimuli must be intense enough to saturate the S-potential and (2) they must be prolonged.

Spectral Sensitivity—The spectral sensitivity of the eye can be investigated to identify photopic and scotopic activity; to provide indirect evidence regarding color vision; to help identify the photopigment underlying vision; or as an important component of phylogenetic, comparative, or ecological studies (Armington 1974).

Since early investigators (i.e., Franz 1931) labeled elasmobranchs as nocturnal predators possessing all-rod retinas, the goal of most investigations on spectral sensitivity of elasmobranchs has been to compare the sensitivity of the animal with its type of photoreceptor and habitat. Kobayashi (1962), using the b-wave of the electroretinogram, found the peak sensitivity of *Mustelus manazo* to be at 505 nm. He correlated this with its life in oceanic waters where light of 480–500 nm penetrates most effectively. Like *Mustelus*, the scotopic (dark-adapted) spectral sensitivity of the torpedo *Narke* peaked at 500 nm and did not shift upon light adaptation. The data for *Raja porosa*

were essentially the same; thus, none of these elasmobranchs displayed a Purkinje shift (i.e., shift in spectral sensitivity during light adaptation).

A Purkinje shift was found, however, in the rays *Holorhinus tobijei*, *Dasyatis akajei*, and *Urolophus fuscus*. The maximum scotopic sensitivity of these rays occurred at 500 nm except for *Dasyatis*, which peaked at 525 nm. Light adaptation shifted the maximum sensitivity of *Holorhinus* and *Dasyatis* to 575 nm. *Urolophus* shifted to 525 nm. These results were correlated with the depth in which the animal was found and its behavior.

Using S-potentials as an index of sensitivity, Tamura et al. (1966), Tamura and Niwa (1967), and Niwa and Tamura (1975) measured spectral sensitivity in a number of elasmobranchs (Table 5). These results were correlated with the presence of a duplex retina. Of the elasmobranchs examined, only *Dasyatis* possessed chromatic S-potentials. The authors attributed this to the possession of cones, which were said to be absent in the sharks examined.

However, *Ginglymostoma*, a close relative of *Orectolobus*, possesses a large number of cones in addition to rods (Hamasaki and Gruber 1965, Wang 1968). It is therefore surprising that cones as well as the associated C-type S-potentials were not found.

The spectral sensitivity of *Mustelus canis* could be described by a single curve peaking at 500 nm regardless of state of adaptation (Dowling and Ripps 1971, 1972; Stell et al. 1970, 1971, 1975). Cones have been found in this retina, but the absence of a Purkinje shift probably reflects their low number and the predominance of rods (Stell and Witkovsky 1973*a*, 1973*b*).

Using the lemon shark *Negaprion brevirostris*, which possesses rods and cones in the ratio of 12:1, Cohen et al. (1977) found a peak scotopic sensitivity at 530 nm. This did not correlate with the λ_{max} of the visual pigment (Bridges 1965*b*) and was shown not to be due to preretinal absorption or to the influence of the tapetum lucidum. It was suggested that the cones were operating under scotopic conditions and contributing to the scotopic spectral sensitivity. In addition, upon moderate light adaptation a shift in the peak spectral sensitivity to 544 nm was also demonstrated. Thus a Purkinje shift is characteristic of the retinal activity of this animal.

Adaptation—The eyes of vertebrates can detect stimuli over a great range of light intensities. This ability to change thresholds with different light levels is termed "adaptation." The changes in threshold can be monitored electrophysiologically to determine the rate and range of adaptation, which can shed light on the mechanisms that control adaptation.

Dark-adaptation curves obtained by monitoring the ERG after exposure of the retina to a background-adapting stimulus was first done on the dogfish *Mustelus* and several rays by Kobayashi (1962). In these experiments changes in threshold amounted to only 2–3 log units and reached a stable level after only 10 min. In addition, only smooth dark-adaptation curves were obtained, with no evidence of any rod-cone breaks in spite of the presence of both rods and cones in the retina of at least one of the rays.

In contrast, Hamasaki and Bridges (1965) and Hamasaki et al. (1967) reported changes of 6 log units in dark-adaptation experiments on lemon

sharks, nurse sharks, and the stingray *Dasyatis sayi*. The curves were characterized by an initial sudden drop in threshold, followed by a slower return to the original dark-adapted thresholds 70 min later. They also reported the absence of a rod-cone break in the dark-adaptation curves in spite of the presence of cones in all three species examined. Although Hamasaki and his coworkers used the b-wave as a measure of sensitivity, they did not really know what the relationship of this transient to the actual sensitivity of the retina was.

To determine this, Dowling and Ripps (1970) compared the adaptational properties of the b-wave to ganglion cell discharges, since the ganglion cells represent the final output of the retina and thus reflect its final sensitivity. They demonstrated that the slopes of increment thresholds were the same for both the ERG (b-wave) and ganglion cell discharges over a 6 log unit range of stimuli. These increment thresholds required long periods to reach a stable value (up to 40 min) and did not seem to saturate, a result unlike that obtained for man (Aguilar and Stiles 1954). In addition, strong light adaptation, which bleached about 80% of the photopigment, suppressed both ganglion cell spikes and b-wave responses for 10 to 15 min. Thereafter, thresholds for both fell rapidly to within 3 log units of the dark-adapted values. Both types of responses then followed the same course of recovery, which lasted 2 h. After 20 min in the dark the time course of these responses was almost identical to that of rhodopsin regeneration. These results were comparable to those from similar experiments on other vertebrates and demonstrated that the early part of adaptation is mediated by neural mechanisms while later adaptation is photochemically controlled (Dowling 1963).

Thus, Dowling and Ripps (1970) concluded that the b-wave of the ERG and the ganglion cell spikes show adaptation properties that are similar and therefore that amplitude of the "b-wave provides a reliable measure of retinal sensitivity in the skate" (p. 512).

During these experiments it was shown that an ERG suppressed by moderate light adaptation will reappear after 10 min in the dark. As stated, very bright adapting stimuli inhibited responses for 15 min, and stable response levels were not reached for an additional 20 min. A similar "silent period" was observed for the ganglion cells. Because the a-wave, which originates in the photoreceptors, also disappears, Dowling and Ripps suggested that the mechanism behind the silent period occurs in the receptors. As expected, a similar silent period occurs for the S-potential. When light is first turned on, the horizontal cell hyperpolarizes to a fixed level (i.e., saturates) and remains at this level for many minutes, during which further responses cannot be evoked. After 5 min the membrane potential starts depolarizing towards its dark-adapted level. Responses of increasing amplitude can be evoked as the membrane potential becomes more positive. Increment thresholds were obtained even upon backgrounds that bleached more than 95% of the available rhodopsin. In fact, increment thresholds for horizontal cells could be measured on background fields 10 000 times (or 4 log units) more intense than those needed to saturate the S-potential. There seemed to be no correlation between the level of the membrane potential and sensitivity. This

suggested to Dowling and Ripps that factors controlling sensitivity occurred not in the horizontal cells but in the photoreceptors.

With present techniques, the photoreceptors of elasmobranchs are too small to record from. Thus it is not possible to directly test the various hypotheses on retinal sensitivity. However, the addition of Na aspartate to the retina suppresses the ERG except for the a-wave, whose distal or leading edge originates at the photoreceptors.

Treating the skate retina with aspartate, Dowling and Ripps (1972) investigated adaptation properties of the photoreceptors by recording the receptor potential. The adaptation properties of the receptor potentials were similar to those of b-wave, S-potential, and ganglion cell responses. These included an initial silent period, induced by a fairly strong background light, which eventually recovered. Thereafter responses could be elicited in the presence of a background light, even though a large fraction of the visual pigment was bleached. Thus, threshold responses assumed a linear relation with background intensity.

In addition to the adaptation response properties of the skate retina previously mentioned, evidence for a gain control mechanism was found. It has been suggested that a background light causes a maintained receptor potential (Boyton and Whitten 1970). This potential or voltage has the effect of moving the receptor towards its saturating voltage, thereby compressing its dynamic range. This gain mechanism therefore opposes the so-called compression effect by increasing the gain of the receptors. Green et al. (1975) recorded receptor potential, S-potential, b-wave, and ganglion cell responses under light and dark adaptation and incremental stimulation to determine the exact site of adaptation in the skate retina. Two sites of adaptation were found: one probably resides in the photoreceptors and operates under intense background light; the second resides somewhere proximal to the horizontal cells and is active during weak background light. This is the site of "network adaptation" which is a loss of b-wave and ganglion cell sensitivity under very dim background lights. It is not seen in increment thresholds of receptors or S-potentials. The b-wave and S-potential reacted differently to a weak adapting light. Therefore, it stands to reason that the site of this network adaptation is not the horizontal cells since, if it were, the S-potential and b-wave would behave alike. In addition, when a weak adapting light was used the receptors and horizontal cells recovered their sensitivity together, while the ganglion cells followed the recovery of the b-wave.

Green et al. (1975) hypothesized that an excess of a substance, perhaps potassium, was responsible for the loss of b-wave sensitivity, since the time course for both b-wave and ganglion cells to reach final threshold levels was several minutes, too slow to be of neural origin.

This hypothesis was further strengthened by Dowling and Ripps (1976), who applied different concentrations of external potassium in the eyecup preparation of the skate. When the data were normalized, increasing the amount of external potassium caused the V log I curve of the b-wave to be shifted to the right on the intensity axis, indicating a loss of sensitivity. No loss of sensitivity was seen for the receptor potential.

Pharmacology—The current hypothesis concerning the mechanism of synaptic transmission between photoreceptors and second-order retinal neurons is that a chemical transmitter is continuously released in the dark, thus depolarizing the horizontal cells. Light causes a reduction in the release of the transmitter, which in turn hyperpolarizes the membrane. Two important questions are (1) What is the evidence supporting this hypothesis? and (2) What chemical transmitters are involved?

Dowling and Ripps (1973) tested this hypothesis by bathing the skate retina in Ringer's solution containing magnesium. It is known that when Mg is applied in high concentrations to chemically mediated synapses, transmission is blocked (Katz and Miledi 1967). Fifteen to 25 s after placing a drop of 100 mM MgCl on the skate retina, the horizontal cell membrane hyperpolarized while light-evoked ERGs and S-potentials decreased in amplitude. After 3 min the membrane potential had dropped to -60 mV and neither ERGs nor S-potentials could be evoked. The effect of Mg is reminiscent of that of intense light stimulation and agrees with the supposition that a transmitter is continuously released in the dark, thus maintaining the horizontal cells in a partly depolarized state. The effects of Mg Ringer's lasted for about 30 min, after which membrane potentials returned to their normal levels.

After application of Mg Ringer's, the b-wave of the ERG disappeared, leaving the a- and c-waves. As stated, the leading edge of the a-wave originates at the photoreceptors while the c-wave derives from the pigment epithelium. Thus the Mg Ringer's affected those synapses proximal to the photoreceptors.

Detailed examination of the waveform showed that the rise time of the leading edge of the a-wave was reduced. Since the b-wave seems to originate in glial cells (Miller and Dowling 1970), its disappearance suggests that the aspartate affects retinal elements internal to the receptors. Intracellular recordings from horizontal cells support this contention, since application of aspartate to the retina depolarizes the membrane (horizontal cell). This is accompanied by an increasing hyperpolarization to light, which diminishes and eventually disappears. When both b-wave and S-potential were monitored simultaneously, their decrease in amplitude followed the same time course.

Ripps et al. (1976), using horseradish peroxidase (HRP), have provided further evidence that continuous release of transmitter in the dark is reduced when the receptors are stimulated by light. The dark-adapted retina of the skate takes up HRP in the cells and processes of the outer plexiform layer. Upon light adaptation, there was a marked reduction in the amount of HRP found in this layer. Adding Mg also reduces the concentration of HRP in the outer plexiform layer. This is expected, since Dowling and Ripps (1973) showed that Mg blocks transmission between chemically mediated retinal synapses and would therefore also block the uptake of HRP.

The amount of HRP reaction product was "somewhat greater" in eyecups treated with Na aspartate than in the controls. This is what would be expected of a putative transmitter that depolarizes the membrane.

Cervetto and MacNichol (1971), using the amino acids L-Na aspartate, L-Na glutamate, and GABA, recorded the intracellular responses from the horizontal cells of the skate retina. In concentrations of 20 mM the horizontal cell membrane potential depolarized until finally the response to light stimuli disappeared. Of the three chemicals tested, only aspartate increased the amplitude of the hyperpolarizing response to light. Glutamate and GABA progressively depolarized the cell membrane and decreased the hyperpolarizing response to light.These results are consistent with the hypothesis of transmitter release in the dark.

Behavioral Studies of Vision

Psychophysics—The first behavioral experiment using visual cues for conditioning a shark was that of Clark (1959). She rewarded adult lemon sharks for pressing a submerged plywood target with their snouts. Under these operant methods the animals eventually associated the target with food and ultimately became conditioned to discriminate between a series of targets, indicating their choice by bumping the "correct" stimulus. Since then several studies using the methods of operant conditioning have been accomplished (see Graeber's article elsewhere in this volume). Among these have been Wright and Jackson (1964) and Aronson et al. (1967), who trained lemon and nurse sharks, respectively, on a visual discrimination task. Aronson et al. first demonstrated that sharks are capable of discriminating targets of differing brightness under an environmentally controlled situation, but neither study quantified the visual results. In addition Aronson et al. showed that sharks learn brightness discrimination about as rapidly as teleosts and rats under similar conditioning situations.

Tester and Kato (1966) attempted to condition black tip, *Carcharhinus melanopterus*, and gray sharks, *C. menisorrah*, to discriminate between various targets. Unlike the previous studies which used food reward, their method involved avoidance conditioning. As a result of their somewhat unorthodox procedures, conditioned responses were erratic and led to an imprecise criterion of discrimination. In addition, their subjects were unable to consistently discriminate between more than 50% of the targets, which included such tasks as square vs triangle, gray vs yellow, and bright vs dim. Because of this, Tester and Kato's (1966) conclusions were very tentative.

Qualitative species differences to the training situation were reported, the black tips being described as more erratic. However, the subjects readily discriminated between rectangles oriented at 90° to each other. Other tests of form discrimination were mostly negative. In view of Graeber's results we must accept Tester and Kato's suggestion that the apparently poor form vision of sharks actually reflected problems related to the experimental methods.

Results of a brightness discrimination test by Tester and Kato indicated that the subjects could distinguish between gray targets differing by only two Munsell units; color-vision tests were inconclusive.

We have been investigating the limits of brightness discrimination in the lemon shark. Again using operant techniques, we trained sharks with food reinforcement to swim to the brighter of two doors illuminated by an array of optical fibers connected to a light source (Figure 21). Under this test situation sharks successfully discriminated as long as the brightness of one door exceeded that of the other by 0.3 log units. Using the identical stimuli, human subjects under aerial viewing were slightly better than the sharks (by 0.1 log units) at choosing the brighter door.

To our knowledge the only psychophysical studies in which visual thresholds were measured and resulting visual parameters generated are those of
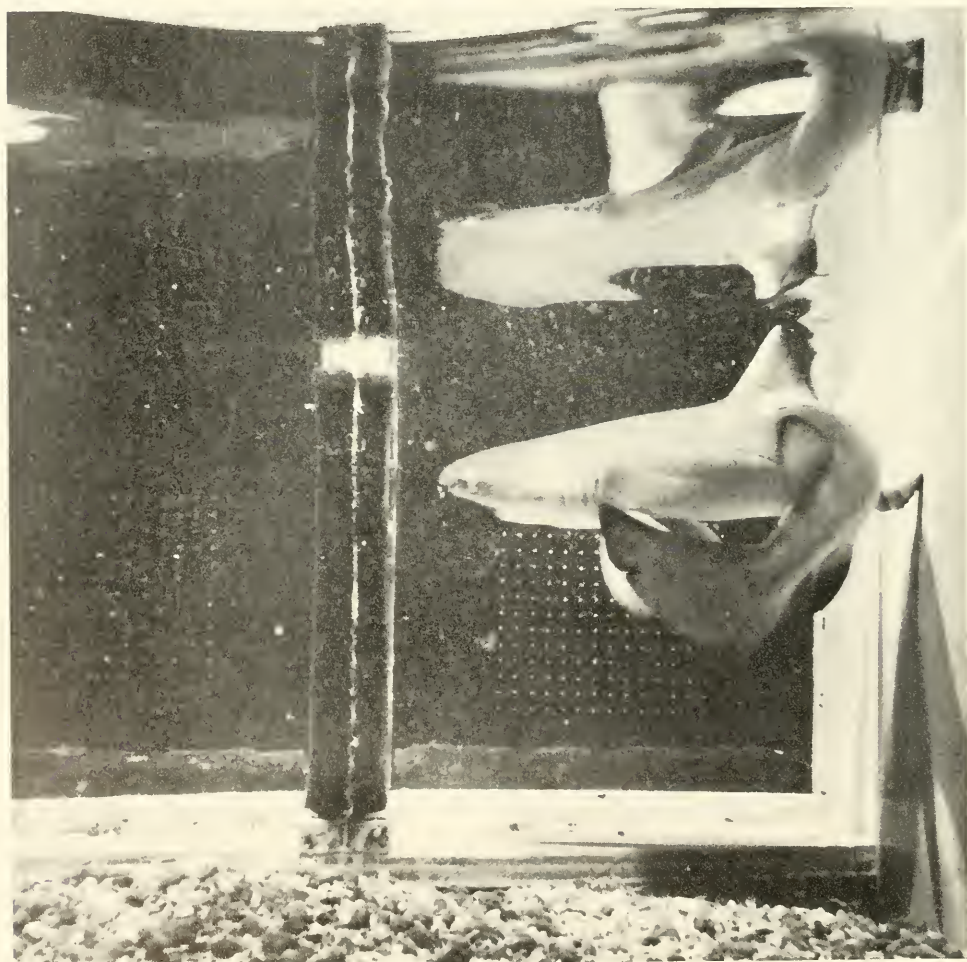
Figure 21   Underwater photograph of *Negaprion brevirostris* at the choice point of a single-choice maze. The shark chooses the brighter stimulus obtained by differentially illuminating 3136 optical fibers whose ends are embedded in one of two guillotine doors. The shark receives food reinforcement behind the correct door. (From Gruber, unpublished experiments.)

Gruber (1966, 1967, 1969, 1975), Cohen et al. (1977), and Gruber and Hamasaki (in preparation). The training technique associated with these investigations has already been described in the previous section on Eyelids. These experiments have been framed in the duplexity theory and seek to provide a relation between receptor type and visual capability of sharks. Duplexity theory assigns separate roles to the photoreceptor systems of the retina: a sensitivity mechanism resides in the rods and a chromatic, acuity mechanism in the cones. Each system operates through a transitional range of light intensities, the cones functioning in daylight, the rods at night. Thus, depending upon which system was in operation, one might expect differences between the visual functions of light- and dark-adapted animals, and at the transition point a discontinuity or break in the smooth curve of a particular visual parameter might signal the changeover from rod to cone control of that parameter. For example, Figure 22 shows how the temporal resolution of various animals increases as intensity of the flashing light
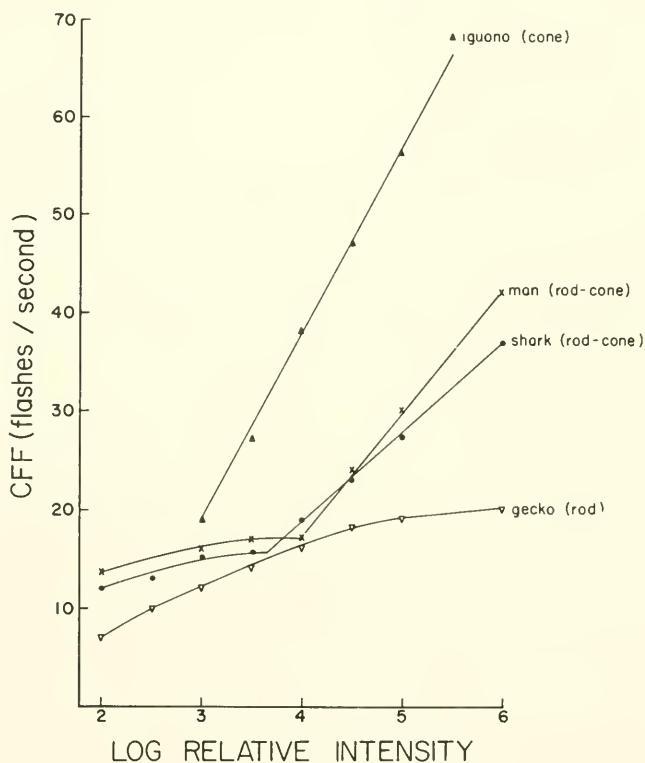
Figure 22   Relation between CFF and stimulus intensity in four vertebrates. The nonhuman data were taken under identical stimulus conditions and are thus directly comparable. The human data are shown for reference only. The curves demonstrate the rod-cone break in duplex retinas compared to the monotonous function of the simplex retina. (From Gruber 1969. Unpublished dissertation.)

increases. A distinct "kink" is seen at about 4 log units; this is associated with the switchover to the cone system. Animals with pure rod or pure cone retinas show a simple or smooth curve. It was this kind of indirect behavioral evidence for cone function that Gruber was seeking. These studies have recently been reviewed, and the reader is referred to Gruber (1975) for details.

Gruber (1966, 1967) first investigated dark adaptation of the lemon shark. Subjects were intensely light-adapted before being placed in darkness. Determinations of the minimum light stimulus detectable by the shark (i.e., threshold of vision) were made as dark adaptation proceeded. Results demonstrated that the lemon shark is capable of extensive slow dark adaptation. Independent electrophysiological investigation (Hamasaki and Bridges 1965) confirmed the results at the retinal level. Infrared photography of pupillary changes during dark adaptation indicated that iris dilation (results discussed in the section Iris) accounted for only tenfold (1 log unit) of the more than 1-millionfold (6 log unit) increase in visual sensitivity accompanying dark adaptation. The final sensitivity of the shark exceeded that of two human subjects measured on the same adaptometer. However, the curve of dark adaptation in the lemon shark was apparently smooth and thus did not provide evidence for a photopic mechanism.

Gruber (1969, 1975) and Gruber and Hamasaki (in preparation) then turned to the parameter of critical flicker fusion. Figure 21 shows the rationale behind the experiment as well as the result. This time sharks were trained to respond when a steady visual field was made to flicker (flicker is a subjective term). The stimulus beam was increased in intensity, and the number of flashes per second which just elicited a conditioned response at each intensity level was recorded. This test revealed a discontinuity or rod-cone break as seen in Figure 22. Concurrent electrophysiological studies confirmed the presence of a rod-cone break in the cff-vs-log I curve. This was the first behavioral evidence favoring cone function in sharks.

The next series of investigations involved spectral sensitivity and the so-called Purkinje phenomenon of the lemon shark (Gruber 1969, 1973, 1975; Cohen et al. 1977). One of the most fully supported relationships in visual science is the correspondence between the absorption characteristics of the (rod) visual pigment and the dark-adapted spectral sensitivity of the animal. Spectral sensitivity may be defined as the minimum number of quanta detectable at each frequency of light within the spectral limits of the particular species. Under dark-adapted conditions the shape of the rhodopsin absorption curve determines the spectral sensitivity of the animal, although ocular features such as a colored lens, oil droplets, or tapetal reflection can affect the curve. As the animal becomes light-adapted a shift in spectral sensitivity may occur, since the rhodopsin (rod) system is suppressed as the cone system whose spectral characteristics are based on other visual pigments becomes active. This shift is named after its discoverer Purkinje and is indirect evidence for cone function.

Figure 23 shows the results of a behavioral determination of spectral sensitivity in the light- and dark-adapted lemon shark. While the dark-adapted

curve agrees in shape and position of maximum sensitivity with the rho-
dopsin absorption curve, the light-adapted shark had somewhat different
sensitivity characteristics. The effect of adaptation with white light caused
the peak of this curve to shift some 20 nm toward the red and broadened the
curve. This shift has been confirmed electrophysiologically (Gruber 1973,
Cohen et al. in press). Thus the Purkinje phenomenon is a feature of the
lemon shark's visual system and is further evidence of cone function in this
species.

Gruber (1969, 1975) described a direct test for color vision in which
lemon sharks were trained to respond when a chromatic adapting field was
silently switched to another color. Controls on stimulus brightness were
included in the test. Statistical analyses of the data indicated that the subject
was using chromatic cues in responding to the stimulus changes. However,
results were presented with reservation since data from only one subject
were available.

Visual Orientations—Harden-Jones (1963) studied the reactions of
various fishes, including the dogfish *Scyliorhinus*, to a rotating striped drum.
Fishes typically responded by swimming around the periphery of the tank
following the movement of the background stripes. Although Hardin-Jones
was interested in the relation of visual cues to orientation in a current, he
was actually measuring what is known as the optomotor response. A number
of species including *Scyliorhinus* failed to respond to the moving background
and thus did not exhibit an optomotor response.

Wallace (1972) reported on the reaction of sharks to gill nets in con-
nection with a meshing program along South African beaches. Although nets
are successful at protecting beaches, it was not known exactly how the nets
act as barriers or how sharks react to them. In one test, Wallace presented
bull sharks, *Carcharhinus lucas*, with nets of various reflectances and colors.
Results indicated that sharks avoided the brighter colored (yellow) nets, thus
Wallace suggested that avoidance of nets in the experimental situation was
based on visual cues.

Other workers have suggested that sharks, in the field, are attracted to or
avoid brightly colored objects. For example, Hess (1962) reported that
oceanic sharks are naturally attracted to fluorescent orange objects. Some-
what more meaningful was the report by McFadden and Johnson (1971)
that survival gear painted yellow was attractive to free-ranging sharks while
the same gear painted black was apparently ignored by the same animals.
This report is reminiscent of a preliminary field study (Gruber, unpublished
results) in which silky sharks, *C. falciformis*, were acoustically attracted to
an array of three polyethylene globes 40 cm in diameter. The globes were
separated by 6 m. In this study, sharks clearly avoided the fluorescent orange
globe but readily removed bait from a black globe and less frequently from a
white globe. Again, this is similar to a study by Limbaugh (1963) in which
baits were placed in fluorescent orange or white bags. In this case, sharks
preferred the white over the orange target, striking the white bag three times
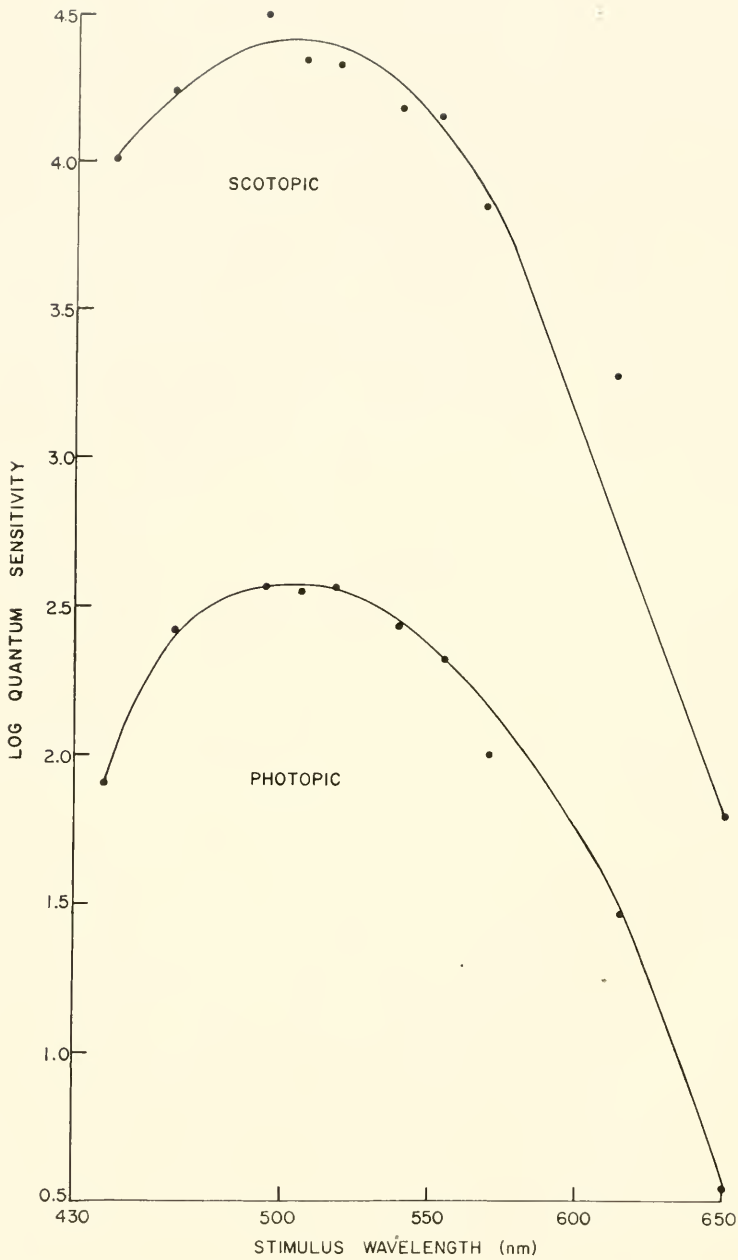
Figure 23 Comparison of light- and dark-adapted spectral sensitivity in *Negaprion brevirostris*. These psychophysical results, confirmed electrophysiologically, show that the sensitivity of this animal shifts upon light adaptation, providing evidence for duplex visual function. (From Gruber 1969. Unpublished dissertation.)

more frequently. The relevance of such studies to visually oriented natural behavior of sharks is questionable.

An important consideration in the behavior of an animal is how ambient changes in illumination affect its activities. It has often been reported that sharks are strictly nocturnal, remaining quiescent during the day and feeding at night (Walls 1942, Bigelow and Schroeder 1948, Randall 1968). Nelson and Johnson (1970), Nelson (1974), and Finstead and Nelson (1975) have provided experimental evidence in three shark species that favors this view. Laboratory observation of activity (swimming) rates under controlled illumination demonstrated that horn sharks, *Heterodontus francisci*, and swell sharks, *Cephaloscyllium ventriosum*, are distinctly nocturnal. Both species possess a drifting endogenous rhythm under constant dim illumination and are strongly inhibited from swimming by bright light. Both are immediately active in darkness, and one horn shark remained continously active for 264 h of darkness. These results were confirmed by field observation and experimentation and extended to the angel shark, *Squatina californica* (Standora 1972), and blue shark, *Prionace glauca* (Sciarrotta 1974).

Observations on other species indicate that gray sharks (Carcharhinidae) are crepuscular, feeding primarily at dawn and dusk (Hobson 1968). Still other species are active by day and feed in shallow water on the brightest afternoon (Starck 1968). Again species differences must account for part of the variation in behavior associated with light levels. However, the data on life history and behavior of sharks are so fragmentary that it is difficult to draw a definite conclusion as to whether sharks are nocturnal, as they have been traditionally labeled. Based on the definitive work of Nelson and his colleagues, however, it is clear that some species are truly nocturnal although they will feed during daylight if given the opportunity.

MEDIAN EYE

The pineal organ has long been suspected of being a light receptor, and in some lower vertebrates the related parapineal organ is even known as the parietal eye. The elasmobranchs possess a well-developed pineal organ, which was described in the early 20th century. Studnicka (1905) reported the presence of ganglion cells, and Holmgren (1918) described inner and outer segments of sensory cells in the pineal parenchyma of *Squalus*. In his noteworthy study, Holmgren homologized the pineal receptors with light-sensitive cells of the retina and demonstrated the presence of nerve fibers in the wall of the pineal organ. Contemporary anatomical studies on the median eye have sought to characterize both ultrastructure and function of this organ.

Anatomy

Altner (1965) reported on histology and histochemistry of the pineal in *Etmopterus*, *Galeus*, and *Squalus*. He described sensory cells in the pineal of

each but was unable to confirm the presence of ganglion cells. Rüdeberg (1968), in a preliminary paper, described the ultrastructure of the pineal receptors in *Scyliorhinus*. In this first analysis of the shark pineal under electron optics, Rüdeberg identified the sensory cells as photoreceptors and more specifically as receptors of the cone type. This criterion for classification followed that of Nilsson (1964): all the outer segment disks were broadly connected to the plasma membrane. Disks of the outer segment of rods, in contrast, have this connection only at their basal portion (Figure 10). In a followup paper (Rüdeberg 1969) the pineal of *Scyliorhinus* was carefully studied under light and electron optics. Rüdeberg recognized six cell-types comprising the pineal parenchyma, including photoreceptors and ganglion cells (few). One ganglion cell appeared to project dual tracts to the posterior and habenular commissures of the brain. Evidence of synapses between receptors and ganglion cells was seen in the form of pre- and post-synaptic vesicles and synaptic rods. An improvement in fixation demonstrated unequivocally that these receptors were of the retinal cone-type. Not only was the plasma membrane of the outer segment broadly connected to the saccules, the saccules themselves displayed a conspicuous lumen characteristic of retinal cone and lacked the type of fissure which gives the rod saccules their typical bilobed appearance. Thus, Rüdeberg concluded, quite correctly, that the pineal of *Scyliorhinus* is photosensitive.

While the pineal appears to be light sensitive, Rüdeberg (1969) noted that *Scyliorhinus* ". . . does *not* have a pineal window or any other adaptation for light reception of the pineal organ" (p. 573). Gruber et al. (1975) took exception to this statement, demonstrating that three elasmobranchs (*Negaprion*, *Carcharhinus*, and *Mustelus*) definitely possess a morphological differentiation of the region directly over the end vesicle of the pineal organ. Photometric measurements indicated that seven times more light impinged on the pineal receptors than on surrounding areas of the brain. It appears that the shark's chondrocranium is modified for transmission of light into the epiphysis.

Physiology

The only physiological study of the elasmobranch pineal apparatus appears to be that of Hamasaki and Streck (1971). They recorded from the epiphysis of *Scyliorhinus* by placing an electrode on the cut end of the epiphyseal stalk transected near the brain. Their objective was to determine whether the pineal was sensitive to light and if so to describe some physiological properties of the pineal system. Stimulation of the epiphysis by a 1-s flash of white light evoked a slow, positive potential lasting up to 15 s. This was accompanied by prompt inhibition of spike activity. The authors investigated the effects of stimulus intensity and steady illumination on pineal activity. They also measured the spectral sensitivity of the system, obtaining a curve which was similar to the rhodopsin curve (i.e., peaking at 500 nm) but too narrow to fit precisely. They then presented evidence indicating that the hemoglobin of the blood surrounding the epiphysis acted as a light filter

and thus narrowed the spectrum impinging on the photoreceptors. They therefore concluded that the pineal receptors contained rhodopsin.

Sensitivity determinations indicated that the pineal system responds to changes of illumination on the order of 4×10^{-6} lm/m^2 which is far below moonlight. Actually the sensitivity of the pineal approaches that of the rod system of the lateral eye. It is difficult to understand why the epiphyseal photoreceptors are morphologically conelike yet behave like rods and possess rod visual pigment.

CONCLUDING REMARKS

The increasing importance of the elasmobranch visual system as an object of study has been made abundantly clear in this article. We were astonished by the number and variety of recent investigations on the eyes of cartilaginous fishes. Objectives for studying the elasmobranch visual system varied from phylogenetic and systematic considerations through use of the shark eye as a simple model of the human eye.

From comparison with the older literature, it was clear that a quantum increase has been achieved in available information on the elasmobranch visual system. This has radically altered our views on structure, function, and adaptation of that system. For example, Duke-Elder (1958) reviewed the elasmobranch eye and listed six characterizing features. We now know that three of these—(1) sluggishly mobile iris without nerve supply; (2) shallow anterior chamber without annular ligament or Schlemm's canal; (3) retina, with few exceptions, provided only with rods—are no longer correct.

While the eyes of the approximately 250 species of cartilaginous fishes share many features in common, there is so much variation that it is difficult to describe the "elasmobranch eye."

Elasmobranchs swim in nearly all major habitats in the marine environment, from the deepest abyssal zones to the littoral surf zone. Pelagic and benthic species are known from Arctic to tropical waters and a few species are even restricted to freshwater. Obviously this ecological diversity will be reflected by adaptations of their organ systems. Compounding this is the wide variation in behavior; some species are apparently highly dependent on vision while others seem to use tactile, olfactory, or other nonvisual cues more frequently. Thus we are presented with an array of elasmobranch visual systems in which the diameter of the eye varies from nearly 10% of the standard length in the big eye thresher, *Alopias superciliosus*, to less than 1% in a large nurse shark, *Ginglymostoma cirratum*. Some sharks have fixed eyelids while others possess a completely mobile nictitating membrane. Certain species have rapidly constricting irides; in others, the iris is nearly immobile. Variability in pupil shape characterizes the elasmobranchs. Some species can even "stop" the pupil down to one or more stenopaic apertures. Many elasmobranchs have a partially or totally occlusable tapetum but the tapetum of *Scyliorhinus*, for example, is fixed. And so the list can be continued. Thus it is difficult to meaningfully define the average "elasmobranch eye."

In general, physical and chemical properties of seawater have profoundly influenced the evolution of the aquatic eye. Differences between water and air with respect to absorption and scattering of light, pressure variations with depth, and frictional forces have led to large differences between the typical aerial and aquatic eye. In aquatic visual systems the eye of elasmobranchs differs considerably from that of bony fishes, for example, in mode of accommodation, pupillary mobility, ocular adnexa, and tapetal structure. It was perhaps Walls (1942) who first realized the peculiar status of the elasmobranch eye. He was surprised to note that several characteristics such as mobile eyelids, ciliary folds, and a flattened lens drawn back from an arched cornea, so typical of the aerial eye, were also found in the shark eye. He thus wrote "we might conclude, from a cursory examination of the shark eye, that the elasmobranchs must once have lived on land and, like whales, secondarily returned to the ocean. Surely these peculiarities all have explanations . . . but we cannot be sure at present that we quite know all the answers" (p 429). This statement is no less true today, 35 years later. In fact, it has been strengthened by newer examples of similarities between the elasmobranch eye and that of "higher" vertebrates, especially mammals.

Two things we can say with some assurance: (1) these unique ocular features reflect the long and independent phylogenetic history of the cartilaginous fishes and (2) the elasmobranch eye cannot be considered as "primitive." Rather, the shark's visual system is housed in a specialized and apparently well adapted vertebrate eye. Clearly, however, the adaptive significance of the ocular components and visual capabilities of elasmobranchs remains unknown. This is partly because knowledge of the natural history, ecology, and behavior of elasmobranchs is confused and fragmentary and partly because it is conceptually difficult to design a direct field study for determining the biological significance of vision. For example, it is not clear whether the typical shark is an active predator, relying on vision to capture prey, or a scavenger. The question of periods of peak activity has not yet been resolved. Is the typical shark nocturnal? To answer these and other questions, studies on ecology and behavior of sharks must be undertaken. Results will certainly aid in interpreting the structural and functional characteristics of the shark visual system.

One final point concerns the relation of vision to the natural behavior of elasmobranchs. Occasionally authors have attempted to correlate visual parameters with ecological and behavioral characteristics of sharks but these have not been very convincing. We now know something of the visual capacities of several elasmobranchs from laboratory studies, and speculation on natural visual behavior is possible. For example, the absolute sensitivity of the lemon shark, *Negaprion*, would permit visual activity under dim moonlight provided hydrographical conditions were favorable. What is unknown is whether these animals actually use that capacity. Thus, a real need exists to study the activities of elasmobranchs under natural or field conditions so that insight might be gained on the importance of the visual system in the lives of the elasmobranchs.

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REFRACTION AND ACCOMMODATION
OF THE ELASMOBRANCH EYE

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INTRODUCTION

Three areas of concern have dominated the comparative study of visual optics: (a) measurement of refractive error, or the degree to which the focal point of light entering the eye fails to coincide with the retina, (b) accommodation, or the ability of the eye to vary its focusing power to maintain image quality for varying fixation distances, and (c) the study of the individual optical components of the eye, including intraocular distances, radii of curvature, and refractive indices.

The optical quality of the elasmobranch eye has been the subject of only five published articles. Moreover, this minute body of literature is remarkably contradictory.

LITERATURE

Beer

Beer (1894) included a small section on elasmobranchs in his voluminous study of the teleost eye. He noted that the mobility of the elasmobranch iris makes measurement of refractive error difficult. In specimens in which a measurement was possible, several diopters of hyperopia were reported. Beer's findings were made with the direct ophthalmoscope, an instrument he assumed was limited by reflection from the vitreous surface of the retina. All final values for teleosts include a compensatory subtraction based on the thickness of the retina. This procedure produced characteristic findings of myopia and led to a controversy concerning the refractive nature of the teleost eye (Verrier 1928; Sivak 1974b, 1975a). Beer did not report whether the same considerations were involved in his determination of hyperopia in elasmobranchs.

Beer also attempted to induce accommodative refractive changes by electrically stimulating enucleated elasmobranch eyes. In the teleost eye, such simulation caused the lens to move toward the retina. No change was noted in elasmobranchs. Beer did not rule out the possibility of an accommodative ability in this vertebrate class. However, he relegated vision to the position of a secondary sense in elasmobranchs and considered olfaction dominant.

Franz vs Verrier

Aside from a brief report by Franz (1905) concerning a failure to electrically induce lens movement, the first studies directed specifically at the optics of the elasmobranch eye were those of Verrier (1930) and Franz (1931). Both commented at length on the difficulties encountered in refracting elasmobranchs. These include (a) the mobility of the pupil and (b) the interposition of large pectoral fins between the eye and an aquarium wall in the case of skates and rays. The retinoscope was used in both studies, although Franz also verified his findings with an ophthalmoscope. In each

case, refractive measurements were made after several minutes of dark adaptation, to permit sufficient pupil dilation.

With skates and rays, Verrier simply removed a portion of one pectoral fin. Franz assumed that the eye in such an instance would appear to lie at a position three-fourths the actual distance between it and the aquarium wall. This factor slightly affected consideration of the distance at which retinoscopy was performed when neutrality of the retinoscopic reflex was achieved. In both studies an average refractive error of 9 or 10 diopters of hyperopia was found.

Verrier (1930) did not verify experimentally whether an elasmobranch accommodative ability exists. She noted, however, that a refractive error of hyperopia precludes a mechanism involving movement of the lens toward the retina, such as that described by Beer (1894) for teleosts.

It is commonly assumed that change in lens shape is not a practical mechanism in aquatic vertebrates because the lens, as the principal refractive structure, has a high refractive index and lacks flexibility (Walls 1942). Reduction in hyperopia can be accomplished only by *increasing* the distance between the lens and retina. Verrier and Rochon-Duvigneaud (1943) denied the existence of an intraocular lens muscle in elasmobranchs. The additional lack of ciliary muscle fibers that might squeeze the globe and elongate the optic axis of the eye (a mechanism suggested by Verrier (1928, 1934, 1947) for teleosts) led both Verrier and Rochon-Duvigneaud to declare that elasmobranchs cannot accommodate.

The only published report indicating that elasmobranchs can accommodate is that of Franz (1931). Franz electrically stimulated the eyes of a number of species after removing the cornea and iris. He observed forward motion of the lens in a ray and in a torpedo but reported little or no success in the case of sharks. Franz believed that the observed lens motion was a result of contraction of ectodermal muscle fibers in a ventral papillary extension of the ciliary body. The question of whether this papilla (the protractor lentis muscle or pseudo-campanule) does in fact contain muscle fibers or whether it is merely a part of the non-contractile suspensory apparatus of the lens is not clear. As mentioned above, Verrier and Rochon-Duvigneaud believed the latter. A histological study by Wang (1968) also disputed the contractile nature of the pseudo-campanule. This controversy may be a result of the inherent difficulty of identifying contractile tissue of ectodermal origin.

The Concept of the "Ramp" Retina

The term *accommodation* as used in the above context refers to a dynamic change in the refractive state of the eye along a specific axis. An additional accommodative concept appears in the literature in respect to rays and horses (Franz 1934; Walls 1942; Duke-Elder, 1958). The term *ramp retina* refers to a static accommodative mechanism consisting of variation of the distance between lens and retina. An animal possessing such an eye would theoretically accommodate for the viewing of near targets by moving the

eyes (or bending the head or body or both) to produce a new and longer visual axis. It is important to emphasize that light rays emanating from such a target will require a longer focal distance (Fig. 1). In effect, this mechanism will produce the same refractive result as one in which the lens moves away from the retina. According to Franz, the asymmetry of the ray eye (*Raja batis*) is such that a transfer of image position from the central to the dorsal retina will fulfill an accommodative function.

PRESENT RESEARCH

Accommodation

The accommodative mechanism involving the protactor lentis has gained widespread acceptance (Prince 1956; Duke-Elder 1958; Tansley 1965). This is because of its acceptance by Walls (1942) and because it tidily relates refractive error (hyperopia) to accommodation. An additional factor is the lack, until very recently, of any subsequent experimentation.

Somiya and Tamura (1973) include some elasmobranchs in a photographic study of lens motion induced electrically in excised teleost eyes. While a lack of movement was reported, it should be noted that the mobility of the elasmobranch pupil may make the position of the lens difficult to record photographically. A recent attempt to induce refractive changes in the eye of the lemon shark by the use of drugs failed as
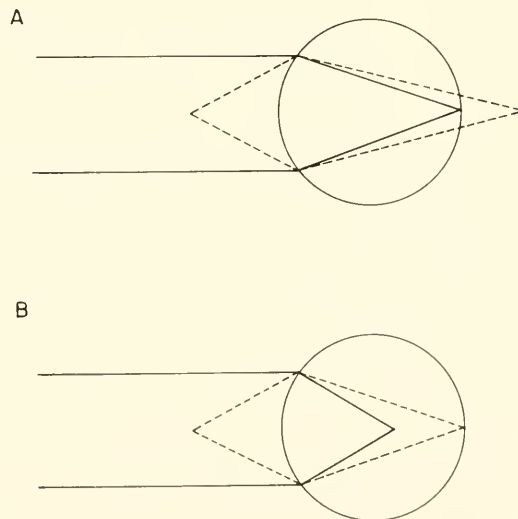
Figure 1   Focal conditions of an eye, indicating that (A) when distant targets are focused on the retina the eye is hyperopic at nearer points, and (B) when near targets are focused on the retina the eye is myopic for distant points.

well (Sivak 1974*a*). However, this failure is tempered by the fact that the drugs chosen were those employed successfully with teleosts (Myer and Schwassmann 1970; Sivak 1973).

In contrast to the above studies, current work (Sivak and Gilbert, 1976) supports the view that sharks can accommodate. This is based on the recording of refractive error differences in nurse and brown sharks (*Ginglymostoma cirratum* and *Carcharinus milberti*) before and after tricaine anaesthesia (Table 1). The direction of the changes suggests movement of the lens toward the retina under anaesthesia. Furthermore, it is possible to reverse the direction of refractive change by electrically stimulating the root of the oculomotor nerve of anaesthetized nurse sharks. Histological study indicates possible contractile elements in the ciliarybody of both species. A report on the accommodative responses of the eye of the bluntnosed stingray (*Dasyatis sayi*) is discussed below.

The Ramp Retina of the Stingray Eye

While a static accommodative mechanism is said to exist in horses and rays, a recent study of the horse eye indicates an almost symmetrical relationship between the lens and retina (Sivak and Allen 1975). However, additional study (Sivak 1975*b*) of the lens-retina relationship in three species of stingrays (dasyatidae) confirms the asymmetry reported by Franz (Fig. 2). In each case the dorsal portion of the retina is further away from the lens than the distance along the geometric axis of the eye. The accommodative effect of this lengthening of the visual axis is about 6 diopters (as determined for *Dasyatis sayi* by calculation and by retinoscopic measurements of refractive error along the appropriate directions).

The same study notes that refractive error varies in *D. sayi* when the unrestrained specimen is presented with flashing targets at varying distances

Table 1. Refractive error changes (right or left eye) in two species of sharks under unanaesthetized and anaesthetized (MS 222) conditions and following electrical simulation of the oculomotor nerve. (+) indicates hyperopia and (−) indicates myopia.*

Species	Unanaesthetized	Anaesthetized	Oculomotor stimulation
Carcharinus milberti	−2.50	0.00	—
	+4.00	+7.00	—
	+3.00	+6.50	—
Ginglymostoma cirratum	−2.00	+8.00	+4.50
	+2.00	+8.25	+4.50
	0.00	+11.00	—

*Sivak and Gilbert, unpublished.
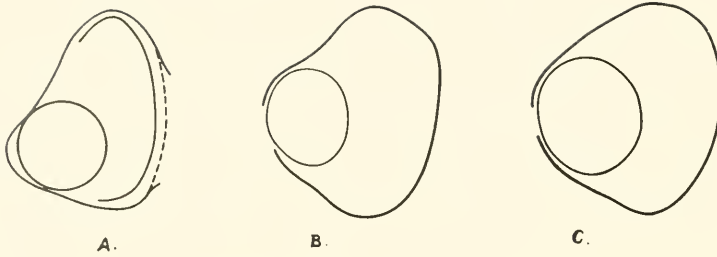
Figure 2   Vertical asymmetry of the ray eye in *Raja batis* (Walls 1942) and *Dasyatis sayi* and *D. Sabina* (Sivak 1975*b*).

from the eye. Refractive error was measured with a retinoscope and trial lenses. Refractive errors became more myopic (or less hyperopic) as target distance decreased, the amount of change corresponding to the difference in the vergence of incident light rays. Since these changes were found along a specific axis of the eye, a dynamic accommodative ability is indicated. In view of the existence of dynamic accommodation, the dorsal lengthening of the visual axis of the eye as an accommodative mechanism must be questioned.

Refractive Components of the Eye

Except for work on the dorsal asymmetry of the ray eye (Franz 1934, Sivak 1975*b*) and comments by Rochon-Duvigneaud (1943) on the asphericity of the elasmobranch lens, the refractive components of the elasmobranch eye have been ignored. Authors such as Wells (1942) and Verrier (1930) have simply extrapolated from the teleosts. For example, they characterize both groups as having spherical lenses of high refractive index. This description, stemming largely from an early study of the optical nature of the teleost eye (Matthiessen 1880), is frequently considered to apply to all aquatic vertebrates.

Preliminary measurements of intraocular dimensions and refractive indices of the ocular media (Sivak 1975*b*; Sivak, unpublished observations) indicate that the optical components of the elasmobranch eye are quite different than those of the teleost (Table 2). Intraocular measurements were made following a rapid freezing and sectioning procedure. All measurements of refractive index were made with an Abbé refractometer. As noted earlier by Rochon-Duvigneaud (1943), the elasmobranch lens is not spherical. For example, the average vertical lens diameter of *Dasyatis sayi* is 18% longer than the horizontal diameter. Refractive indices of elasmobranch lenses appear to be significantly lower than the equivalent value of 1.69 reported for the goldfish (Charman and Tucker 1973). Lens indices, in elasmobranchs, were found by squeezing the lenses between the refractometer prisms, a procedure which is not possible with the teleost lens. The asphericity of the lens and its lower index of refraction mean that incident

Table 2. Comparison of lens shape and average refractive indices of the ocular media in elasmobranchs and teleosts

| Species | Lens shape | Refractive index | | | |
		Cornea	Aqueous humor	Lens (overall)	Vitreous humor
Teleosts					
Carassius auratus[*]	spherical	1.335	1.335	1.69	1.337
Elasmobranchs	vert. diam.				
	18% > horiz.	1.373	1.342	1.481	1.342
Dasyatis sayi[†]					
Ginglymostoma cirratum[‡]	vert. diam.				
	16% > horiz.	1.382	1.342	1.487	1.341

[*]Charman and Tucker 1973.
[†]Sivak 1975*b*.
[‡]Sivak, unpublished observations.

light rays must travel further beyond the lens in order to focus on the retina. This greater lens-to-retina distance is apparent in *D. sayi* (Sivak 1975*b*).

Corneal refractive indices (Table 2) are significantly higher than the value reported for teleosts by Charman and Tucker (1973). This is of little refractive importance, since the radii of curvature of the two corneal surfaces are generally very similar. As long as the refractive index of the aqueous humor approaches that of water, most (if not all) of the refractive effect produced by the external corneal surface will be negated by the posterior one. Thus, the interspecies variation of corneal refractive index assumes importance only when the eye is exposed to air.

DIRECTIONS OF FURTHER RESEARCH

An understanding of the optical capabilities of the elasmobranch eye will require more persistent study than has been carried out thus far. The results of past and present work, as well as the problems to be overcome, are summarized in the following lines.

The question of whether elasmobranchs have any accommodative ability is still unanswered. Efforts to induce accommodative changes have produced more failures than successes. It should be recognized that attempts to induce these changes artifically will not solve the problem unless these attempts are accompanied by study of the natural response. For example, in the above study of the dynamic accommodative response of *D. sayi*, refractive error changes were recorded retinoscopically in response to flashing targets presented at varying distances from the eyes of unrestrained specimens (Sivak 1975*b*). However, the retinoscope is not suitable for recording rapid changes of refractive errors, and other optical methods

should be explored. In teleosts, the visibility of the lens through the cornea permits the use of cinematography (Sivak and Howland 1973). The mobility of the elasmobranch pupil precludes this method, because the lens is not visible.

Accommodation and refractive error are strictly optical terms that provide little behavioral information. However, both are related directly to resolution ability. Schwassmann and Myer (1971) studied accommodation in teleosts by noting the electrophysiological response of the optic tectum to moving fine wires. They determined visual acuity (by the narrowest wire yielding an optimum response) at various distances from the eye, and from this could infer a refractive error. Accommodative information was yielded by drug-induced refractive error changes. This study is an example of a method for determining the optical quality of the eye from its performance in resolving fine detail. Visual acuity may also be found directly, by the use of conventional behavioral experiments. Both classical and operant conditioning methods have been used in studies of the elasmobranch visual system. Gruber (1975) examined such psychophysical functions as dark adaptation and critical fusion frequency of the lemon shark eye by conditioning the excursion of the nictitating membrane. Graeber and Ebbesson (1972) used a food-reward paradigm to study the ability of nurse sharks to discriminate between horizontal and vertical grid targets. Thus, the combination of behavioral acuity measurements with a conventional optical approach appears to be a promising experimental avenue.

As far as the question of refractive error per se is concerned, the literature review presented here indicates a predominance of hyperopia. It has been suggested that the restricted spectral nature of large bodies of water (blue or blue-green), in conjunction with ocular chromatic aberration, could result in spurious findings of hyperopia (Sivak 1974b). Thus, study of the optical components of the elasmobranch eye should include measurement of aberrations. Perhaps the spectral quality of the normal environment should be duplicated during measurement of refractive error.

Finally, optical study of the elasmobranch eye cannot be separated from other considerations of visual performance. Recent evidence of diurnal visual activity in elasmobranchs (Gruber 1975; Gruber et al. 1975) emphasizes the need for an adequate description of the eye as an optical instrument.

ACKNOWLEDGMENTS

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BRAIN ORGANIZATION IN THE CARTILAGINOUS FISHES

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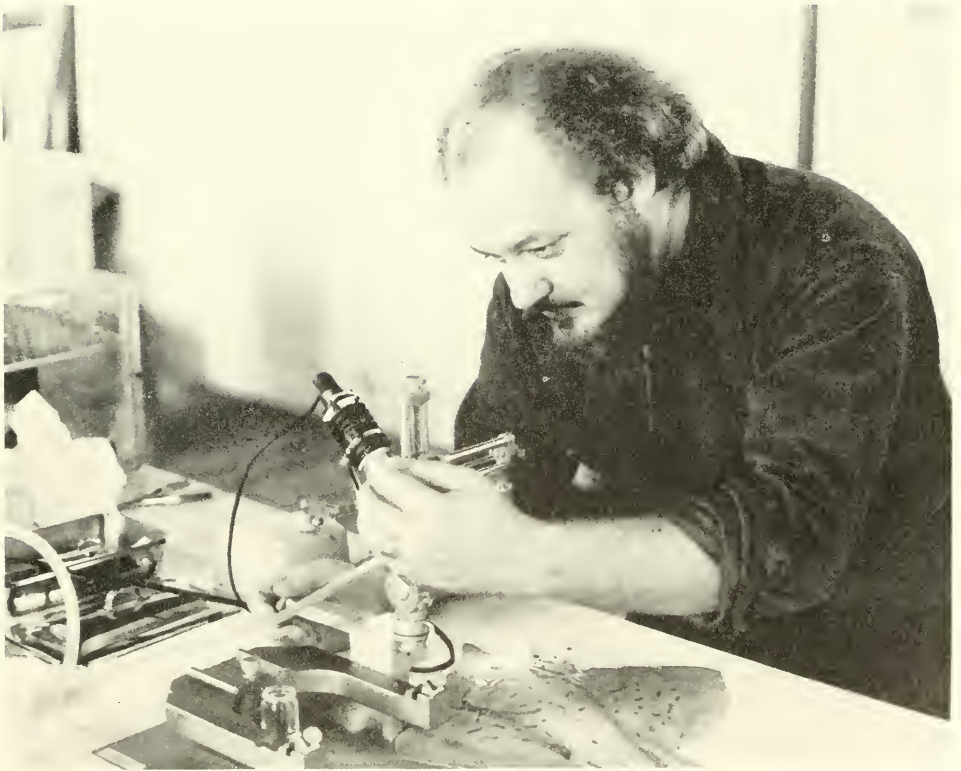
The eyes were sightless in the black, and the other senses transmitted
nothing extraordinary to the small, primitive brain.

<div align="right">Peter Benchley, Jaws, 1974</div>

Instead of a "swimming nose," a picture is beginning to develop of a
shark as a sensory wonder, attuned to sights, sounds, smells, movement,
electrical impulses, and even the movement of the earth. None of
these sensory abilities implies any more intelligence than was previously
attributed to the sharks; we can still assume that they react pretty
much on the basis of instinct. . . .

<div align="right">Richard Ellis, The Book of Sharks, 1975</div>

INTRODUCTION

Until recently elasmobranchs were considered primitive fish with small,
simple brains mediating a behavioral repertoire limited compared to those of
bony fish or land vertebrates. The elasmobranch telencephalon was said to
function primarily in olfaction, and its efferents were believed to project
principally to epithalamic and hypothalamic centers integrating olfactory
and gustatory behavior. The roof of the midbrain was believed to be the
highest visual center—capable of only crude visual analysis—where ascending
somatic and visual sensations were integrated into a few stereotyped
behavioral responses. The well-developed cerebellum was believed to relate
to powerful, well-coordinated trunk movements, yet sharks were said to be
clumsy.

These conclusions are rapidly being replaced by a newer picture of elasmo-
branch central nervous system (CNS) organization. However, it is important
to understand how sharks came to be viewed as primitive, robotlike smelling
and feeding machines. Until the 1950s, most comparative studies were
framed within typological considerations. Nonmammalian vertebrates were
assumed to represent earlier, and thus simpler, stages in the evolution of
mammals. Attention focused on recognizing morphological features common
to all vertebrates (yielding a common pattern or vertebrate *Bauplan*), and on
assigning different vertebrates to different "phylogenetic levels" or "stages."
Elasmobranchs have cartilaginous skeletons, which were believed to predate
bone in vertebrate evolution. Thus, they were assigned to a primitive ("low,"
in typological thinking) position in vertebrate evolution.

The myth was easily perpetuated. Most biologists, as students or re-
searchers, have examined the spiny dogfish (*Squalus acanthias*) or the lesser
spotted dogfish (*Scyliorhinus caniculus*). *Squalus* is representative of less
than 24% of the living sharks, and is a member of the most primitive group
of living sharks (squalomorphs), while *Scyliorhinus* is one of the most primi-
tive members of the advanced sharks (galeomorphs). Examination of either
species gives little idea of the range of morphological complexity exhibited
by elasmobranchs. Even worse, one might assume that all elasmobranchs are
similar to these two taxa. The magnitude of this misconception can be

realized if one can imagine a concept of mammalian morphological variation and general biology based only on rats and armadillos.

Preoccupation with recognition of common morphological features and their phylogenetic levels clearly dominated early comparative neurobiological studies (Papez 1929, Kappers et al. 1936), and such considerations are, regrettably, more evident in contemporary comparative neurobiology (Crosby et al. 1967, Sarnat and Netsky 1974) than in other comparative disciplines. This is perhaps due to the strongly human-oriented nature of much vertebrate neurobiology.

Neural features common to all vertebrates tell us little about specific adaptations, and thus little about the evolution of any vertebrate group. At best, features common to widely divergent species provide clues to the origin and initial adaptations of vertebrates. It is differences in morphological features that signal adaptation and, thus, evolution.

It is equally fallacious to characterize neural features of different vertebrate species as points on a linear, simple-to-complex scale with mammalian neural organization at the acme. Vertebrate evolution is not a unilinear hierarchy, but rather a series of radiations, widely divergent and separated since the early Devonian period. Within each of these radiations, adaptations have continued to occur; and frequently similar, as well as different, solutions have evolved in response to complex environmental forces.

I believe a main focus of comparative neurobiology should be to sample brain variation among living vertebrates and to recognize different morphological patterns and their adaptive significance, rather than reconstructing a unilinear phylogenetic history of vertebrate brains "from fish to man." Only by sampling the existing variation can adaptive patterns be recognized. Vertebrate features are not like the elements of the periodic table—it is impossible to predict what variation should exist based on an incomplete sample, since evolution is opportunistic. Once the sampling is fairly complete, patterns can be recognized and hypotheses about the biological significance of such patterns can be formulated and tested.

For example, several species in two or more vertebrate radiations have independently evolved large brains with separate and complex sensory and motor representations in homologous brain centers. Do these adaptations reflect similar life styles? Perhaps we can recognize certain behavioral and ecological correlates that are always associated with a particular morphological pattern. Perhaps a given pattern has evolved a number of times as the most advantageous pattern (if not the only pattern genetically possible) for any group of vertebrates using the environment in a particular way.

In this chapter, the gross brain variation in cartilaginous fishes is described, and different patterns of neural organization are recognized. Our present knowledge regarding CNS organization in cartilaginous fishes is reviewed. Neural similarities among different groups of cartilaginous fishes are noted, and comparisons are made with other vertebrate groups. Many of the patterns recognized have most likely evolved independently, and their possible adaptive significance is discussed.

MATERIALS AND METHODS

Gross Anatomy

The genera used in this analysis are listed in Table 1. Those that I examined are indicated by an asterisk; published accounts exist for all other genera listed. The taxonomic scheme is that of Compagno (1973, 1977).

Table 1. Chondrichthian Central Nervous Systems described in literature or examined by author.*

Class Chondrichthyes

Subclass Holocephali

Rhinochimaera pacifica
(Garman 1904)
Callorhynchus antarcticus
(Kuhlenbeck and Niimi 1969)
Callorhynchus milii
(Garman 1904)
Chimaera monstrosa
(Sterzi 1905, Johnston 1910, Kappers and Carpenter 1911, Holmgren 1922, Faucette 1969a, 1969b, Schnitzlein and Faucette 1969)
*Hydrolagus colliei
(Garman 1904, Kuhlenbeck and Niimi 1969)

Subclass Elasmobranchii

Superorder Squalomorphii

Order Hexanchiformes
Chlamydoselachus anguineus
(Masai 1961)
Hexanchus
(Kappers et al. 1936)
*Notorynchus maculatus
(Daniel 1934)
Heptranchias
(Johnston 1911, Bäckström 1924)

*Those examined by author.

Table 1. Chondrichthian Central Nervous Systems described in literature or examined by author.* — Continued

Order Squaliformes

   Etmopterus lucifer
   (Okada et al. 1969, Masai et al. 1973)
   Etmopterus hilianus
   Squalus acanthias
   (Sterzi 1905, Johnston 1911, Holmgren 1922, Bäckström 1924,
   Saito 1930, Leghissa 1962, Schnitzlein and Faucette 1969, Smeets
   and Nieuwenhuys 1976)
   Dalatias licha
   (Burckhardt 1907)
   Deania rostrata
   (Okada et al. 1969)
   Centroscyllium ritteri
   (Okada et al. 1969)

Order Pristiophoriformes

   Pristiophorus japonicus
   (Okada et al. 1969)

Superorder Batoidea

   Order Rajiformes

   Rhinobatos productus
   Platyrhinoidis triseriata
   Raja
   (Hoëvell 1911)
   Raja batis
   (Schnitzlein and Faucette 1969)
   Raja clavata
   (Sterzi 1905, Johnston 1911, Bäckström 1924, Leghissa 1962,
   Veselkin 1965)
   Raja eglanteria

   Order Pristiformes

   No known literature

*Those examined by author.

Table 1. Chondrichthian Central Nervous Systems described in literature
or examined by author.* — Continued

Order Torpediniformes

   Torpedo ocellata
   (Sterzi 1905, Bäckström 1924, Hugosson 1955, Leghissa 1962,
   Bruckmoser 1973, Bruckmoser and Dieringer 1973, Platt et al.
   1974)
   Narcine brasiliensis
   (Schnitzlein and Faucette 1969)

Order Myliobatiformes

   Dasyatis americana
   Dasyatis centroura
   Potamotrygon motoro
   Myliobatis aquila
   (Johnston 1911, Kappers et al. 1936)
   Myliobatis californica
   (Daniel 1934)
   Myliobatis freminvillii

Superorder Squatinomorphii

   Order Squatiniformes

   Squatina dumerili

Superorder Galeomorphii

   Order Heterodontiformes

   Heterodontus francisci
   (Daniel 1934)
   Heterodontus japonicus
   (Masai 1962, Kusunoki et al. 1973)

   Order Orectolobiformes

   Ginglymostoma cirratum
   (Ebbesson and Ramsey 1968, Ebbesson and Heimer 1970,
   Ebbesson and Schroeder 1971, Ebbesson 1972, Cohen et al. 1973,
   Ebbesson and Campbell 1973, Schroeder and Ebbesson 1974,
   Schroeder and Ebbesson 1975)

*Those examined by author.

Table 1. Chondrichthian Central Nervous Systems described in literature or examined by author.* — Continued

Order Lamniformes

    Odontaspis
    (Okada et al. 1969)
    Mitsukurina owstoni
    (Masai et al. 1973)
    Alopias
    (Okada et al. 1969)
    Carcharodon carcharias
    (Gilbert 1963)
    Isurus oxyrinchus
    (Gilbert 1963, Okada et al. 1969)
    Lamna
    (Kappers et al. 1936)

Order Carcharhiniformes

    Scyliorhinus caniculus
    (Haller 1898, Edinger 1901, Sterzi 1905, Johnston 1911, Dart
    1920, Bäckström 1924, Beccari 1930, Gerlach 1947, Bruckmoser
    and Dieringer 1973, Platt et al. 1974, Smeets and Nieuwenhuys
    1976)
    *Scyliorhinus retifer
    Scyliorhinus stellaris*
    (Sterzi 1905, Johnston 1911, Bäckström 1924, Leghissa 1962)
    Mustelus canis
    (Shaper 1898, Houser 1901, Bäckström 1924, Gerlach 1947,
    McCready and Boord 1976)
    Mustelus laevis
    (Sterzi 1905, Bäckström 1924, Leghissa 1962, Platt et al. 1974)
    *Triakis scyllia
    Galeocerdo cuvieri
    (Ebbesson and Ramsey 1968)
    Scoliodon
    (Johnston 1911, Masai 1962)
    *Carcharhinus floridanus
    *Carcharhinus leucas
    *Carcharhinus milberti
    *Aprionodon isodon
    Negaprion brevirostris
    (Tester 1963, Graeber and Ebbesson 1972a)
    Prionace glauca

*Those examined by author.

Table 1. Chondrichthian Central Nervous Systems described in literature or examined by author.* — Continued

(Aronson 1963, Okada et al. 1969)
Sphyrna lewini
Sphyrna tiburo
Sphyrna zygaena
(Okada et al. 1969)

*Those examined by author.

Brain:Body Data

Brains of a number of elasmobranch species (Table 2) were perfused or fixed by emersion in AFA.* All specimens were adults based on gonadal tissues and reported adult body lengths. AFA fixation results in an 8–9% reduction in brain weight, and all brain weights reported are uncorrected for this reduction. Reported body weights are from fresh, unfixed material. Additional data from values cited by Crile and Quiring (1940), Quiring (1950), Ridet et al. (1973), and Bauchot et al. (1976) were used, and they are noted in Table 2.

Data for relative development of major brain divisions (Figure 1) were obtained by immersing AFA-fixed brains in fixative and dissecting the following brain divisions for weighing: olfactory bulbs, telencephalon (including olfactory peduncles), diencephalon, mesencephalon, cerebellum, and medulla. The caudal boundary of the telencephalon was considered to be a plane extending from the rostral border of the optic chiasm. The caudal boundary of the diencephalon was considered to be a plane extending from the rostral pole of the optic tectum to the caudal pole of the infundibulum. The optic nerves were not included in the weight of the diencephalon, but were transected within 2 mm of the chiasm. The cerebellum was considered to include all tissue lying dorsal to a rostrocaudal transection just below the ventral lip of the cerebellar auricle. The caudal boundary of the medulla was set at the level of the first complete cervical spinal nerve. All cranial nerves were transected at the base of the brain, and neither they nor the meninges, blood vessels, and chorioid plexus of the fourth ventricle were included in the brain division weights.

Each brain division was blotted immediately before being weighed. A Mettler analytical balance (Model H10) was used for all measurements. The accuracy of 10 repeated measurements on small brain divisions (0.003 g) was ±1.6%.

Histology

The brains of embryos as well as adults were fixed in AFA, dissected from the heads, embedded in paraffin, and sectioned at 15 μm in the transverse

*90 ml 80% ethanol, 5 ml formalin, 5 ml glacial acetic acid.

Table 2. Elasmobranch brain:body data

Species	Brain weight (g)	Body weight (kg)	Encephalization quotient
Carcharhinus obscurus (Bauchot et al. 1976)	20.76	12.00	1.51
Aprionodon isodon	18.75	10.87	1.47
Sphyrna lewini	59.88	55.71	1.38
Carcharhinus falciformis	43.32	36.24	1.37
Odontaspis taurus (Crile and Quiring 1940)	82.55	123.00	1.05
Scyliorhinus caniculus (Ridet et al. 1973)	1.38	0.57	0.99
Mustelus canis	8.31	6.50	0.96
Galeocerdo cuvieri (Crile and Quiring 1940)	107.50	200.00	0.95
Galeocerdo cuvieri (Bauchot et al. 1976)	20.74	23.50	0.91
Carcharhinus leucas	54.36	83.80	0.92
Heterdontus francisci	4.30	2.93	0.90
Ginglymostoma cirratum	31.65	45.30	0.85
Squalus acanthias	3.87	4.20	0.62
Squatina squatina (Bauchot et al. 1976)	2.06	6.00	0.25
Potamotrygon motoro	4.51	0.63	2.77
Dasyatis sabina (Quiring 1950)	76.52	17.58	1.47
Dasyatis pastinaca (Bauchot et al. 1976)	24.87	6.80	1.28
Dasyatis centroura	19.86	5.66	1.24
Myliobatis freminvillii	14.28	5.43	0.93
Rhinobatos productus	9.11	3.62	0.91
Raja eglanteria	1.66	1.10	0.57
Torpedo marmorata (Bauchot et al. 1976)	1.55	1.87	0.31
Platyrhinoidis triseriata	1.37	2.03	0.25

plane. Sections were stained by Bodian silver impregnations, cresyl violet, or Klüver-Barrera methods. Brain sections illustrated in Figures 2 through 7 are from late fetal stages of *Squalus* (9.5-cm snout-vent length), *Mustelus* (13.5-cm snout-vent length), and *Platyrhinoidis* (neonate). Individuals at this stage of development possess all cell groups recognizable in the adults, but the cell groups and their boundaries are not obscured by subsequent 24-fold brain enlargement.

Weight of brain subdivisions as percent of total brain

	Hydrolagus	Squalus	Etmopterus	Mustelus	Scyliorhinus	Isurus	Prionace	Carcharhinus	Sphyrna	Platyrhinoidis	Raja	Rhinobatos	Myliobatis	Dasyatis
OLFACTORY BULBS	7	6	9	14	14	3	3	3	7	7	3	5	11	8
TELENCEPHALON	31	24	29	32	35	37	45	51	52	27	32	35	39	47
DIENCEPHALON	6	7	9	7	7	6	8	8	4	8	7	8	5	5
MESENCEPHALON	14	15	10	11	12	18	13	8		11	13	10	9	
CEREBELLUM	21	20	16	16	15	19	13	13	6	14	17	14	20	8
MEDULLA	21	28	27	20	17	17	18	17	12	33	28	28	16	14

Figure 1 Relative development of major brain divisions in a number of cartilaginous fishes: *Hydrolagus colliei* (ratfish), *Squalus acanthias* (spiny dogfish), *Etmopterus hilianus* (blackbelly dogfish), *Mustelus canis* (smooth dogfish), *Scyliorhinus retifer* (chain dogfish), *Isurus oxyrinchus* (mako shark), *Prionace glauca* (blue shark), *Carcharhinus milberti* (sandbar shark), *Sphyrna lewini* (scalloped hammerhead shark), *Platyrhinoidis triseriata* (thornback skate), *Raja eglanteria* (clearnose skate), *Rhinobatos productus* (guitarfish), *Myliobatis freminvillii* (bullnose ray), *Dasyatis centroura* (roughtail stingray).

In addition to examining the histology of a number of embryo and adult brains, I also used experimental methods to determine the projections of the olfactory bulb and retina in fetal and adult *Squalus acanthias* and *Mustelus canis* and in adult *Raja eglanteria*. Two techniques were used: the Fink-Heimer method for staining degenerating axons and their terminals after experimental lesions and the autoradiographic method for tracing labeled proteins. The technical details of these procedures can be found in Northcutt and Butler (1976) and Northcutt (1976, 1977a).

RESULTS

General Considerations

The cartilaginous fishes comprise at least two groups, the holocephalons (chimaeras) and the elasmobranchs (sharks, skates, and rays). These groups are believed to share a common ancestor (Schaeffer and Williams 1977). The chimaeras, or ratfishes, possess brains (Figure 8) very similar to those of elasmobranchs but clearly distinct from them in a number of neural characters. All chimaeras possess olfactory bulbs arising from the rostral pole of the telencephalon, while the olfactory bulbs of elasmobranchs arise laterally via elongated olfactory peduncles or tracts (Figure 9). In *Chimaera* and *Hydrolagus*, the olfactory bulbs arise directly from the telencephalic hemisphere (Figure 8), while the bulbs of some rhinochimaerids and callorhinchids arise via elongated peduncles (Garman 1904, Kuhlenbeck and Niimi 1969).
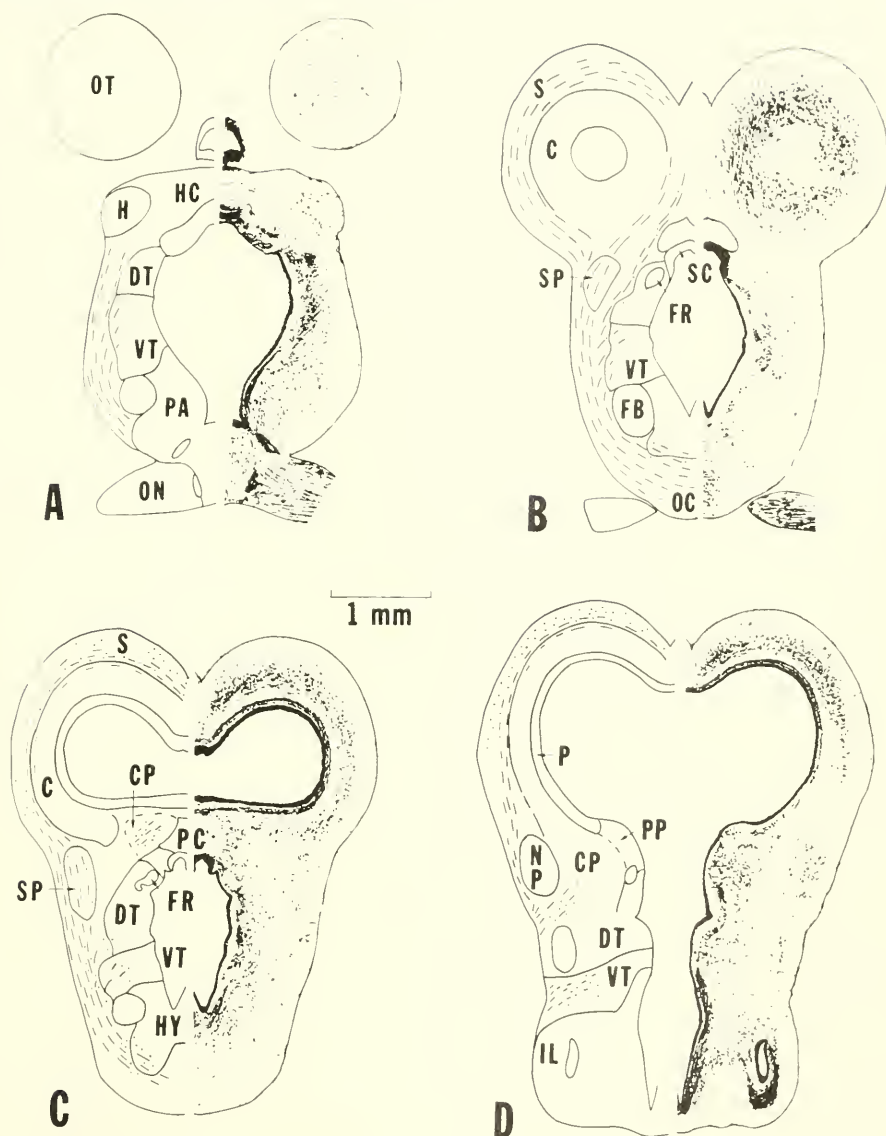
Figure 2 Transverse sections through the diencephalon and mesencephalon of *Squalus acanthias*. In this figure and Figures 3–5, a photograph of a Nissl preparation is shown on the right. To the left is a drawing of the contralateral brain wall charting the course of the retinal projections. Fibers of passage are indicated by dashed lines, and terminal fields by stippling. (A) rostral thalamus, (B) midthalamic level and rostral pole of tectum, (C, D) caudal thalamic levels. C, central tectal zone; CP, central pretectal nucleus; DT, dorsal thalamus; FB, forebrain bundles; FR, fasciculus retroflexus; H, habenular nuclei; HC, habenular commissure; HY, hypothalamus; IL, inferior lobe of hypothalamus; NP, nucleus profundus mesencephali; OC, optic chiasm; ON, optic nerve; OT, optic tectum; P, periventricular tectal zone; PA, preoptic area; PC, posterior commissure; PP, periventricular pretectal nucleus; S, superficial tectal zone; SC, subcommissural organ; SP, superficial pretectal nucleus; VT, ventral thalamus.
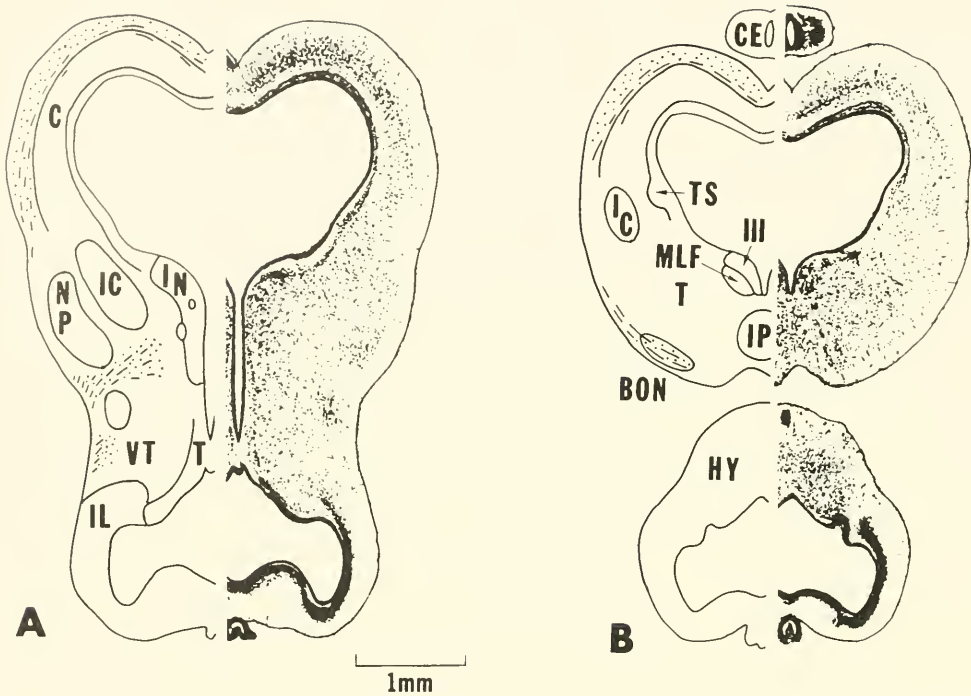
Figure 3   Transverse sections through (A) rostral and (B) midmesencephalic levels of
Squalus acanthias. BON, basal optic nucleus; C, central tectal zone; CE, corpus of cere-
bellum; HY, hypothalamus; IC, intercollicular nucleus; IL, inferior lobe of hypothalamus;
IN, nucleus interstitialis; IP, interpeduncular nucleus; MLF, medial longitudinal fascic-
ulus; NP, nucleus profundus mesencephali; T, tegmentum; TS, torus semicircularis; VT,
ventral thalamus; III, oculomotor nucleus.

The chimaeras lack pallial formations bridging the two telencephalic hemi-
spheres (Figures 9, 10)—a feature that characterizes all living elasmobranchs
(Northcutt 1977b). Chimaeras may also lack a specialization of the telen-
cephalic roof, termed a central nucleus, that is characteristic of all elasmo-
branchs. However, it is also possible that they possess a similar but
independently derived pallial specialization. The confusion results from the
lack of experimental details concerning telencephalic organization in
chimaeras and will be discussed in more detail in a subsequent section.
Finally, all chimaeras possess a specialized elongated telencephalon medium
(tm, Figure 8) whose length may be almost half that of the entire brain.
    The exact intergroup relationships among elasmobranchs are in dispute
(Compagno 1977), but four distinct groups can be recognized: squalomorphs
(hexanchiform, squaliform, and pristiophoriform sharks), batoids (skates and
rays), squatinomorphs (angel sharks), and galeomorphs (some 73% of the
living sharks, including lamniforms and carcharhiniforms).
    Sharks exhibit a wide range of brain variation, but this variation generally
reveals two major patterns of organization. Hexanchiform, squaliform,
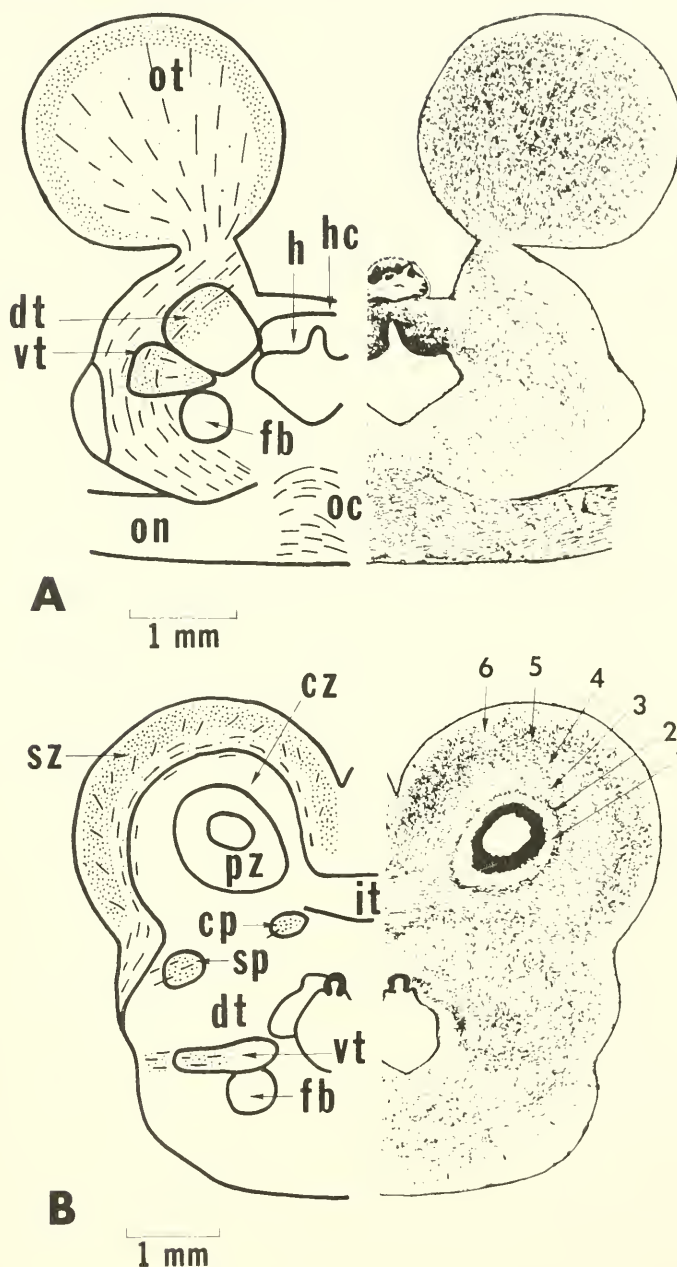
Figure 4 Transverse sections through (A) rostral and (B) caudal thalamic levels of *Mustelus canis*. cp, central pretectal nucleus; cz, central pretectal zone; dt, dorsal thalamus; fb, forebrain bundles; h, habenular nuclei; hc, habenular commissure; it, intertectal commissure; oc, optic chiasm; on, optic nerve; ot, optic tectum; pz, periventricular tectal zone; sp, superficial pretectal nucleus; sz, superficial tectal zone; vt, ventral thalamus; 1–6, tectal layers of Gerlach.
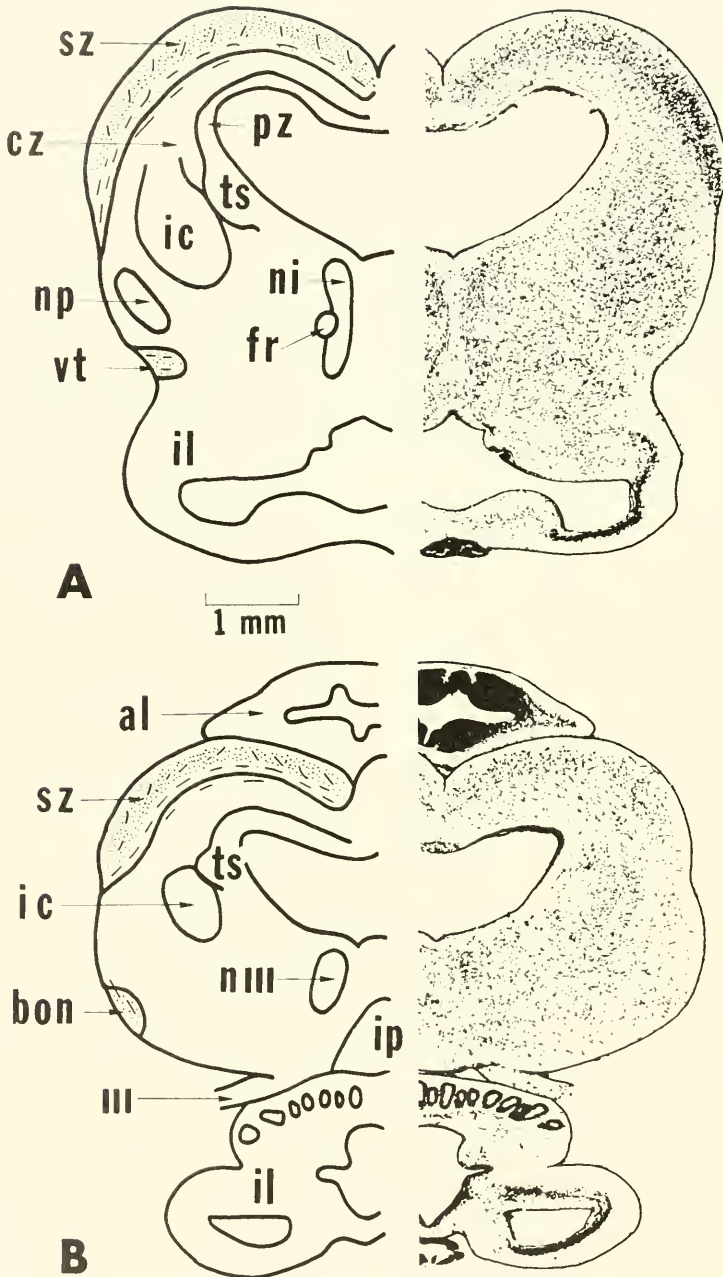
Figure 5 Transverse sections through (A) rostral and (B) midmesencephalic levels of *Mustelus canis*. al, anterior lobe of cerebellum; bon, basal optic nucleus; cz, central tectal zone; fr, fasciculus retroflexus; ic, intercollicular nucleus; il, inferior lobe of hypothalamus; ip, interpeduncular nucleus; ni, nucleus interstitialis; np, nucleus profundus mesencephali; nIII, oculomotor nucleus; pz, periventricular tectal zone; sz, superficial tectal zone; ts, torus semicircularis; vt, ventral thalamus; III, oculomotor nerve.
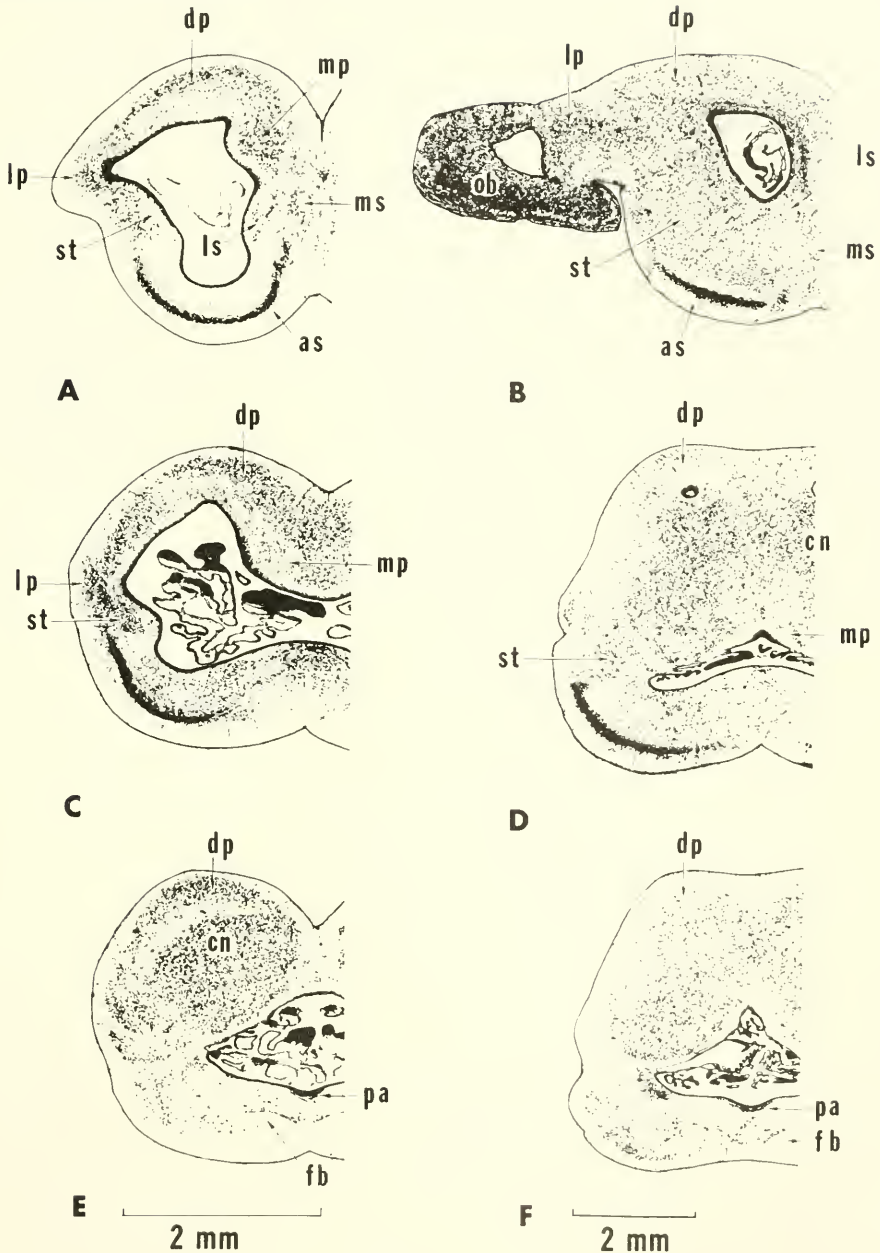
Figure 6   Photomicrographs of comparable transverse sections through rostral, mid, and caudal levels of the right telencephalic hemishpere of (A.C.E) the spiny dogfish, *Squalus acanthias*, and (B.D.F) the smooth dogfish, *Mustelus canis*, illustrating the reduction in lateral ventricles and marked hypertrophy of the roof (pallial) neural groups that occur in carcharhiniform sharks. as, area superficialis basalis; cn, central nucleus; dp, dorsal pallium; fb, forebrain bundles; ls, lateral septum; lp, lateral pallium; mp, medial pallium; ms, medial septum; ob, olfactory bulb; pa, preoptic area of hypothalamus; st, striatum.
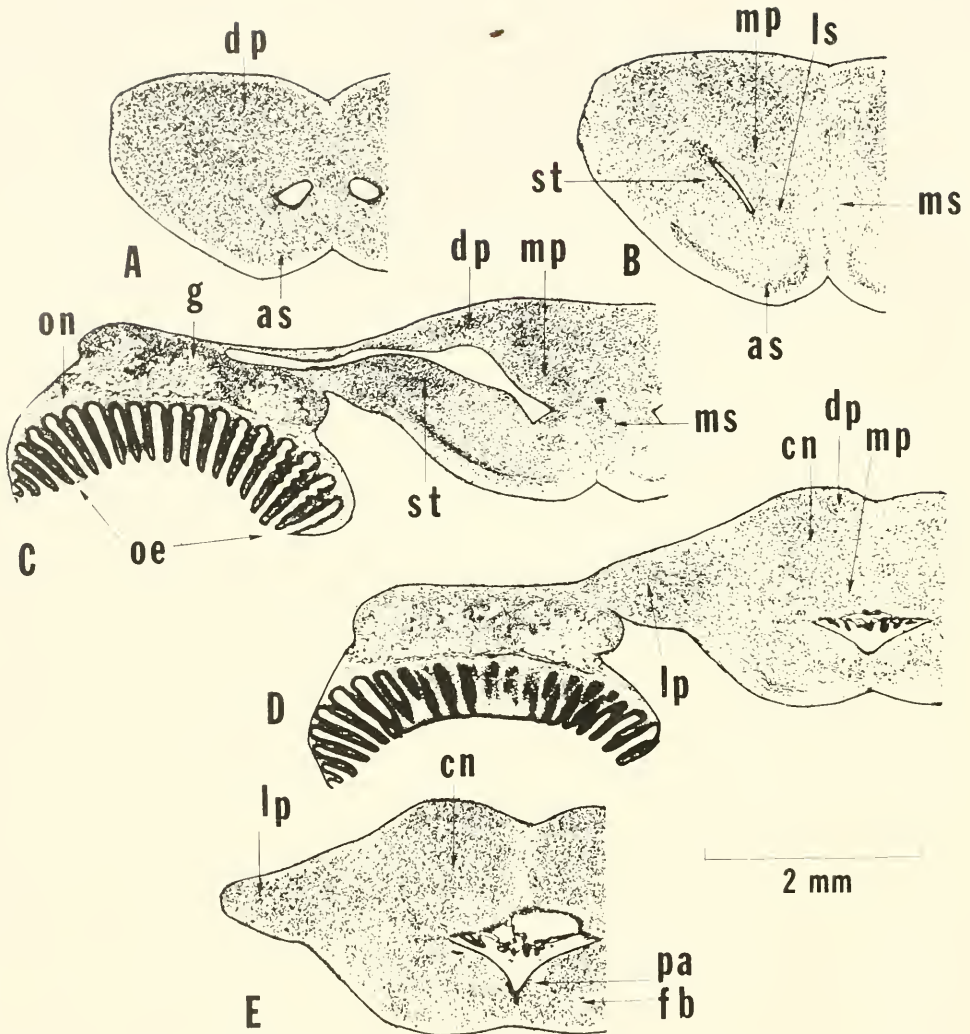
Figure 7 Transverse sections through the right telencephalic hemisphere and olfactory organ of the thornback skate, *Platyrhinoidis triseriata*. as, area superficialis basalis; cn, central nucleus; dp, dorsal pallium; fb, forebrain bundles; g, glomerular layer of olfactory bulb; lp, lateral pallium; ls, lateral septal nucleus; mp, medial pallium; ms, medial septal nucleus; oe, olfactory epithelium; on, olfactory nerve; pa, preoptic area; st, striatum.

pristiophoriform, and squatinomorph sharks have a nonconvoluted corpus of the cerebellum, a well-developed and dorsally exposed optic tectum, a diencephalon with few migrated nuclei, and a poorly developed telencephalon (Figures 2, 3, 9–11). This brain pattern will be referred to here as the squalomorph pattern.

In contrast, galeomorph sharks have a convoluted corpus of the cerebellum with hypertrophy leading to asymmetry in many families (orectolobids, lamnids, carcharhinids, and sphyrnids), an optic tectum

Figure 8   Dorsal view of the brain of the chimaera, *Hydrolagus colliei*. alll, anterior
lateral-line lobe; b, buccal ramus of anterior lateral-line nerve; dob, dorsal division of
olfactory bulb; il, inferior lobe of the hypothalamus; oe, olfactory epithelium (organ); ot,
optic tectum; p, deep ophthalmic or profundus nerve; plln, posterior lateral-line nerve; so,
superficial ophthalmic rami of anterior lateral-line and trigeminal nerves; th, telencephalic
hemisphere; tm, telencephalon medium; vob, ventral division of olfactory bulb; II, optic
nerve; V, mandibular and maxillary rami of trigeminal nerve; VII, facial nerve; VIII,
statoacoustic nerve; IX, glossopharyngeal nerve; X, vagal nerve.

overlapped by the cerebellum and characterized by hypertrophy of the super-
ficial tectal zone, extensive migrated diencephalic nuclei, and hypertrophy of
the telencephalon (figures 4–6, 12–14). This brain pattern will be referred to
as the galeomorph pattern.

Exceptions to these general trends do occur. Scyliorhinids have the com-
plex telencephalic and tectal development characteristic of the galeomorph
pattern, but their cerebellum is unconvoluted, like that of squalomorph
sharks. *Heterodontus*, the horned shark, is a problematic taxon. Its general
brain form suggests close affinity to squalomorph sharks, with which it has
frequently been grouped. However, Compagno (1973) believes *Heterodontus*
is closely related to the galeomorph orectolobids. Details of its neural
organization are lacking, so it is impossible to determine whether the
resemblance to squalomorph sharks is more than superficial.

All batoids possess complex telencephalic and diencephalic organization
similar to that of galeomorph sharks. Cellular migration and thickening of the
telencephalic wall reduces the lateral ventricles to mere vestiges (compare
Figure 10 to Figure 15). Rajiforms and torpediniforms have a simple or
slightly convoluted cerebellum (Figures 11, 16), while the myliobatiforms
have an independently evolved large brain size and a complexly convoluted
cerebellum (Figures 1, 11, 17) whose asymmetry parallels that of carcharhinid
and sphyrnid sharks.
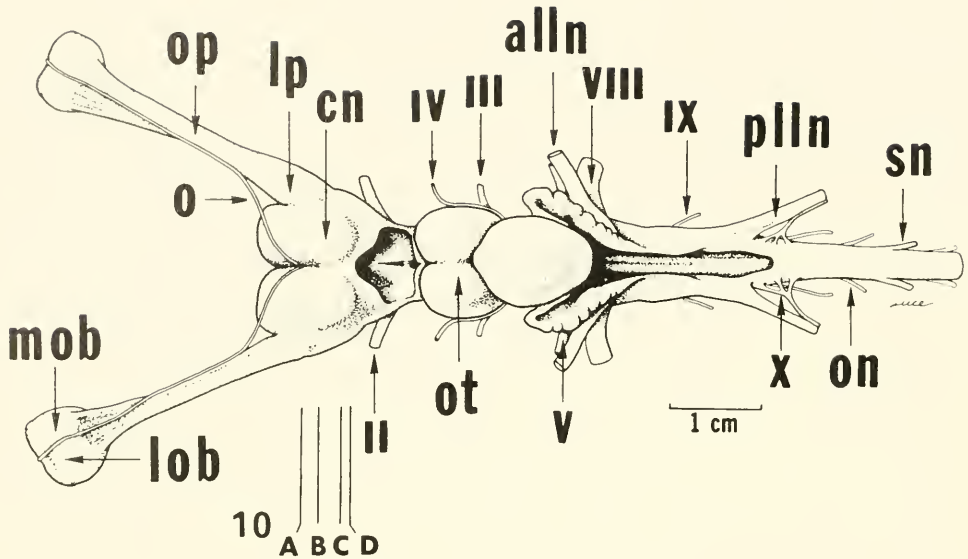
Figure 9   Dorsal view of the brain of an hexanchiform shark, *Notorynchus maculatus*. alln, anterior lateral line nerve; cn, central nucleus; lob, lateral division of olfactory bulb; mob, medial division of olfactory bulb; lp, lateral pallium; o, terminal nerve; on, occipital (hypoglossal) nerve; op, olfactory peduncle (tract); ot, optic tectum; plln, posterior lateral line nerve; sn, spinal nerve; II, optic nerve; III, oculomotor nerve; IV, trochlear nerve; V, trigeminal nerve; VIII, statoacoustic nerve; IX, glossopharyngeal nerve; X, vagal nerve.

The myliobatiforms and carcharhiniforms are characterized by the most complex neural development among living elasmobranchs. At present, all measures of neural complexity fail to distinguish which of these two groups is more advanced. Clearly each has independently reached a complex level of neural organization, in a beautiful example of convergent or parallel evolution.

Quantitative Brain Data

The difference in brain volume or weight among different radiations is one important measure of brain evolution. Direct comparisons are obviously complicated by the wide range of body sizes encountered among vertebrates. However, data for such comparisons can be obtained by making use of the allometric relation between brain weights and body weights expressed by the equation $E = kP^{\alpha}$, where E and P are brain and body weights, respectively, and k and α are constants.

The logarithmic transformation of the above equation becomes $\log E = \log k + \alpha \log P$. This is a linear equation in $\log E$ and $\log P$ with a slope α termed the coefficient of allometry. This coefficient is a measure of the rate of change in brain weight or volume for a given change in body weight or volume. $\log k$ is the intercept, often termed the index of cephalization.

Gould (1966, 1971) and Jerison (1973) have discussed a number of problems associated with the concept of such an index, one of the most
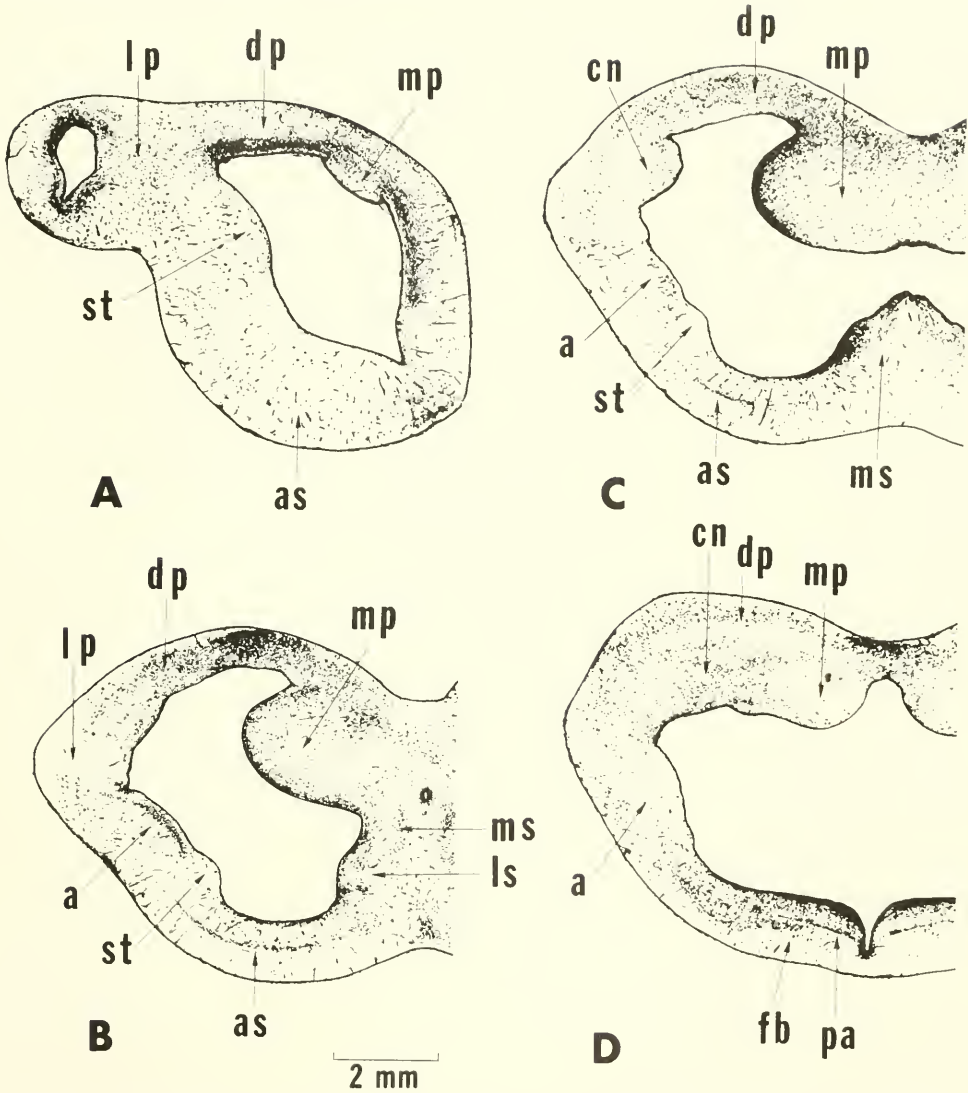
Figure 10 Transverse sections through the right telencephalic hemisphere of an hexanchiform shark, *Notorynchus maculatus*. The levels of these sections are indicated in Figure 9. a, cell group a (possible pallial amygdala); as, area superficialis basalis; cn, central nucleus; dp, dorsal pallium; fb, forebrain bundles; ls, lateral septal nucleus; lp, lateral pallium; mp, medial pallium; ms, medial septal nucleus; pa, preoptic area; st, striatum.

important being that it is a numerical quantity whose biological significance is unknown. Fortunately, Jerison (1970, 1973) has critically reviewed the problem of brain:body indices and has suggested that the philosophy of curve fitting based on the assumption that samples represent random deviations of a true mean (caused by measurement error) should be replaced by a curve-fitting procedure that assumes that the samples represent a region
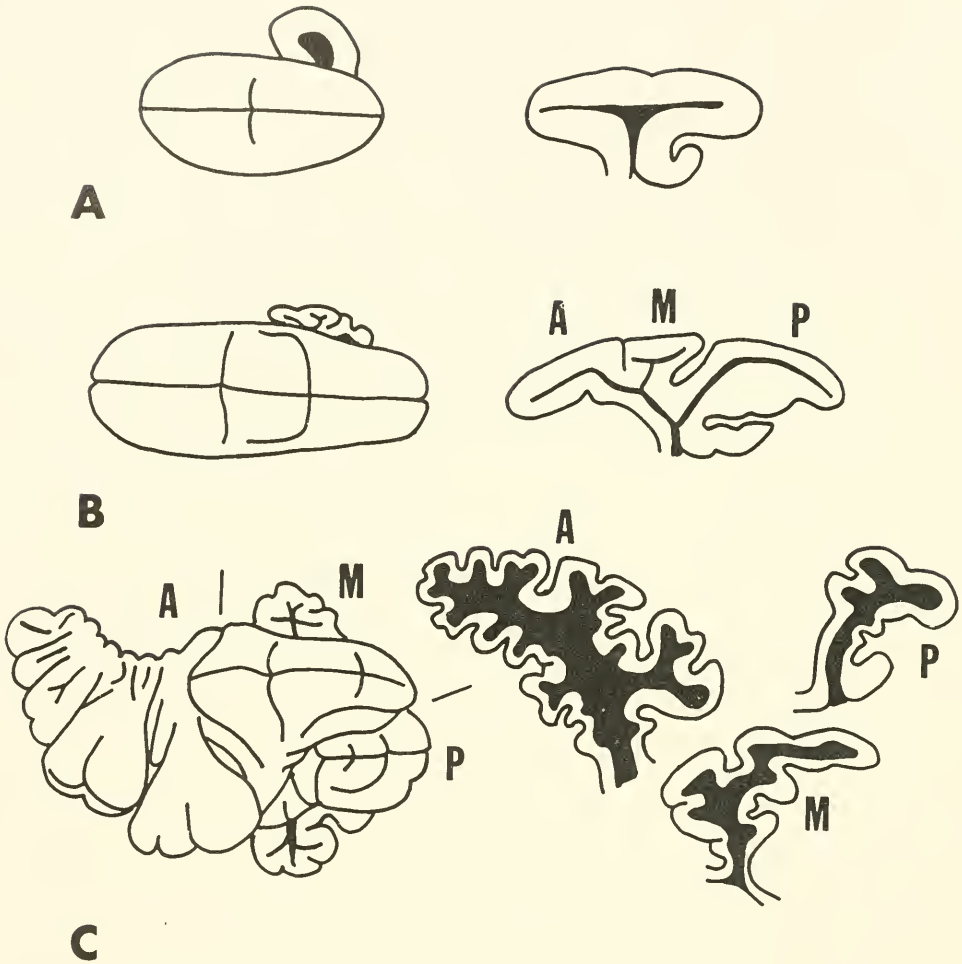
Figure 11   Dorsal and midsagittal views of the cerebellum of (A) *Squalus acanthias*, (B) *Rhinobatos productus*, and (C) *Myliobatis freminuvillii*. Rostral is to the left of the figure. A, anterior lobe; M, middle lobe; P, posterior lobe. Extent of cerebellar ventricle (indicated in black) in *Myliobatis* is greatly distorted due to reconstruction; actual ventricular extent is comparable to other sagittal sections.

within which a set of brain:body data exist for a taxon. Such a region is represented by a principal axis defined for a set of points distributed rectangularly in the area in which they lie. This area and its principal axis can be enclosed by a minimum convex polygon, which then maps the area of the sample set.

Figure 18 illustrates Jerison's (1970) evaluation of the avian, osteichthyan, and mammalian data collected by Crile and Quiring (1940). The stippled polygon encloses the elasmobranch brain:body data reported by Ebbesson and Northcutt (1976) and Northcutt (1977*b*). All elasmobranch data are listed in Table 2, and a detailed plot is presented in Figure 19.
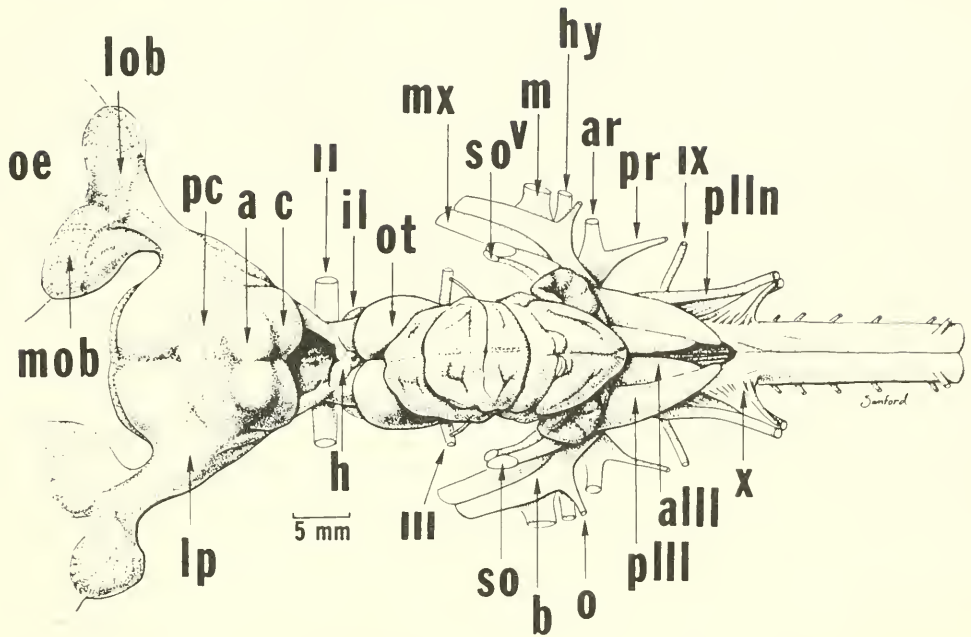
Figure 12 Dorsal view of the brain of a carcharhiniform shark, *Mustelus canis*, the smooth dogfish. a, anterior division of the central nucleus; alll, anterior lateral-line lobe; ar, anterior ramus of statoacoustic nerve; b, buccal ramus of anterior lateral-line nerve; c, caudal division of the central nucleus; h, habenula; hy, hyomandibular trunk; il, inferior lobe of the hypothalamus; lob, lateral division of the olfactory bulb; lp, lateral pallium; m, mandibular ramus of the trigeminal nerve; mob, medial division of the olfactory bulb; mx, maxillary ramus of the trigeminal nerve; o, otic ramus of the anterior lateral-line nerve; oe, olfactory epithelium (organ); ot, optic tectum; pc, pallial commissure; plll, posterior lateral-line lobe; plln, posterior lateral-line nerve; pr, posterior ramus of the statoacoustic nerve; so, superficial ophthalmic ramus of the anterior lateral-line nerve; so^V, superficial ophthalmic ramus of the trigeminal nerve; II, optic nerve; III, oculomotor nerve; IX, glossopharyngeal nerve; X, vagal nerve.

The data in Figures 18 and 19 demonstrate clearly that elasmobranchs possess large brains, comparable in size to many avian and mammalian brains. In addition, the range of variation in brain size is approximately the same for elasmobranchs as for other vertebrate classes.

The extremely high brain:body ratios for elasmobranchs cannot be attributed to the light weight of their cartilaginous skeletons relative to bone. My analysis of *Mustelus canis* indicates that the skelton accounts for 15% of its body weight. This figure is well within the range of skeletal weight percentages reported for other vertebrates (Reynolds and Karlotski 1977). A minimum correction factor of 15-fold would be necessary for elasmobranchs to be placed on the same principal axis as other anamniotic vertebrates in a polygon.

The elasmobranch coefficient of allometry is also very high relative to that for other vertebrates. Based on data reported earlier (Northcutt 1977b), I
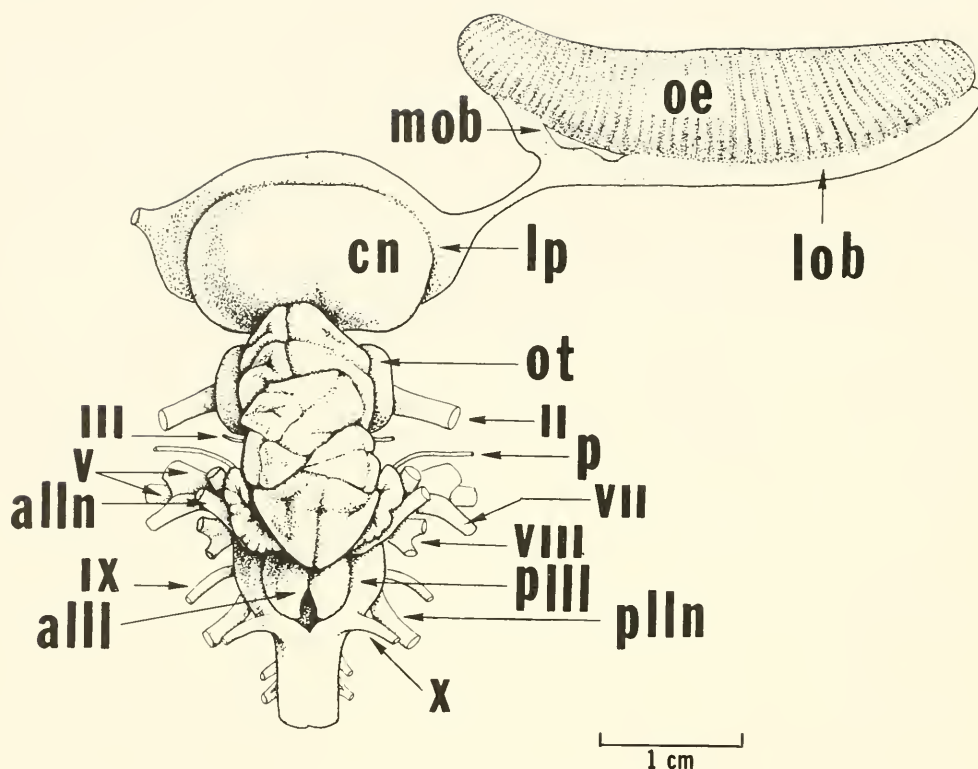
Figure 13   Dorsal view of the brain of a sphyrnid shark, *Sphyrna tiburo* (the bonnethead shark). alll, anterior lateral-line lobe; alln, anterior lateral-line nerve; cn, central nucleus; lob, lateral division of the olfactory bulb; lp, lateral pallium; mob, medial division of the olfactory bulb; oe, olfactory epithelium (organ); ot, optic tectum; p, deep ophthalmic or profundus nerve; plll, posterior lateral-line lobe; plln, posterior lateral-line nerve; II, optic nerve; III, oculomotor nerve; V, trigeminal nerve; VII, facial nerve; VIII, statoacoustic nerve; IX, glossopharyngeal nerve; X, vagal nerve.

determined an overall elasmobranch coefficient of allometry (α) of 0.76, with a coefficient of determination (r^2) of 0.86. The coefficient of allometry for the sharks in this sample is 0.75 ($r^2 = 0.96$), whereas the coefficient for the skates and rays is even higher ($\alpha = 1.04$; $r^2 = 0.67$). Similar elasmobranch data were reported by Bauchot et al. (1976). These workers reported an elasmobranch coefficient of 0.939 but did not calculate separate coefficients for sharks and rays.* Using the data listed in their Table 1, I calculated a shark coefficient of 0.73 ($r^2 = 0.78$), and a skate and ray coefficient of 1.38 ($r^2 = 0.83$). These figures agree closely with my own data, and together they indicate that the batoids possess iso- or positive allometry, a condition rarely

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*An error by Bauchot et al. was incorrectly indicated in Northcutt 1977*b*, a result of misinterpretation in personal communication and failure to realize differences in the respective statistical methods employed.
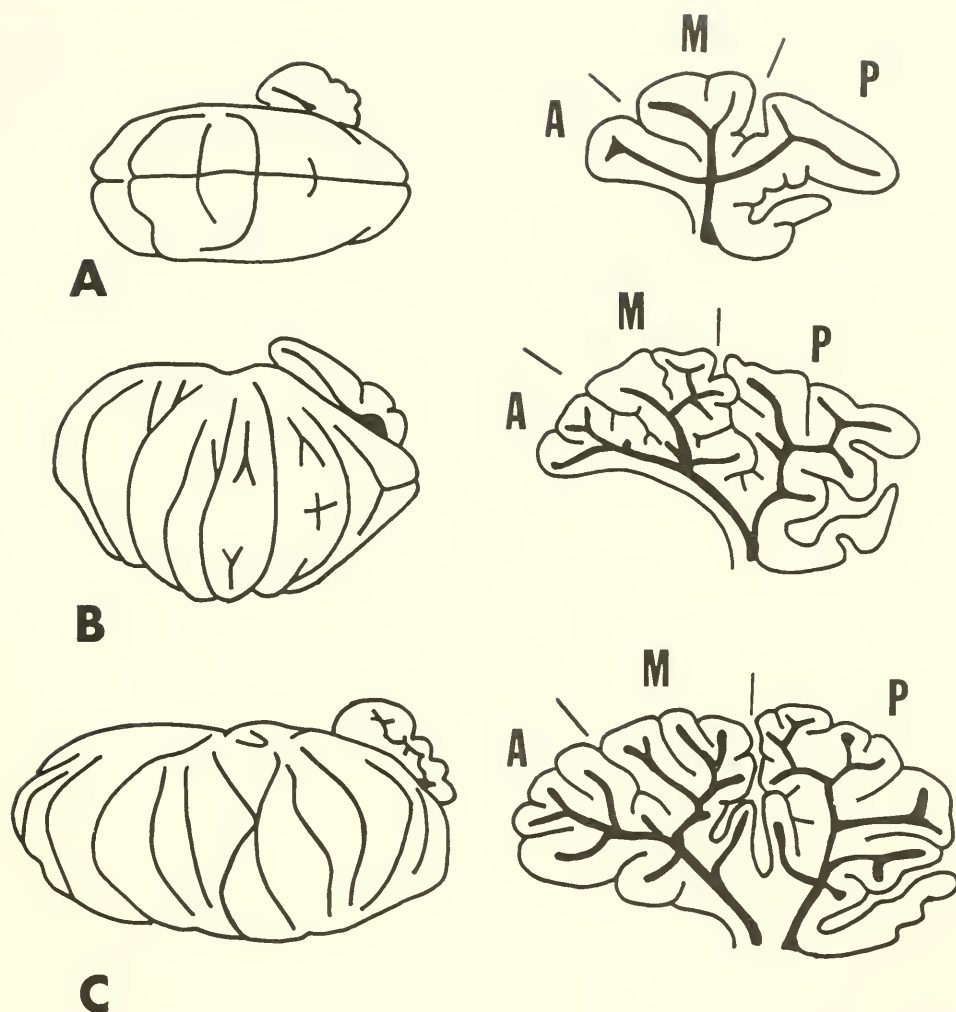
Figure 14 Dorsal and midsagittal views of the cerebellum of (A) *Mustelus canis*, (B) *Prionace glauca*, and (C) *Isurus oxyrinchus*. Rostral is to the left of the figure. A, anterior lobe; M, middle lobe; P, posterior lobe.

reported for vertebrates. Coefficients of allometry range from 0.63 to 0.65 for mammals, 0.56 to 0.60 for amphibians, and 0.65 for teleosts (Bauchot et al. 1976).

The present sample of taxa is too incomplete to suggest trends within the elasmobranchs that may have increased brain size (Figure 19). However, comparison of species of comparable body size, such as *Squalus acanthias*, *Mustelus canis*, and *Sphyrna tiburo*, suggests that the squalomorph sharks may be characterized by low brain:body ratios and that the evolution of the galeomorph sharks is characterized by a twofold to sixfold increase in brain size. Similarly, the batoids range throughout the polygon, but the
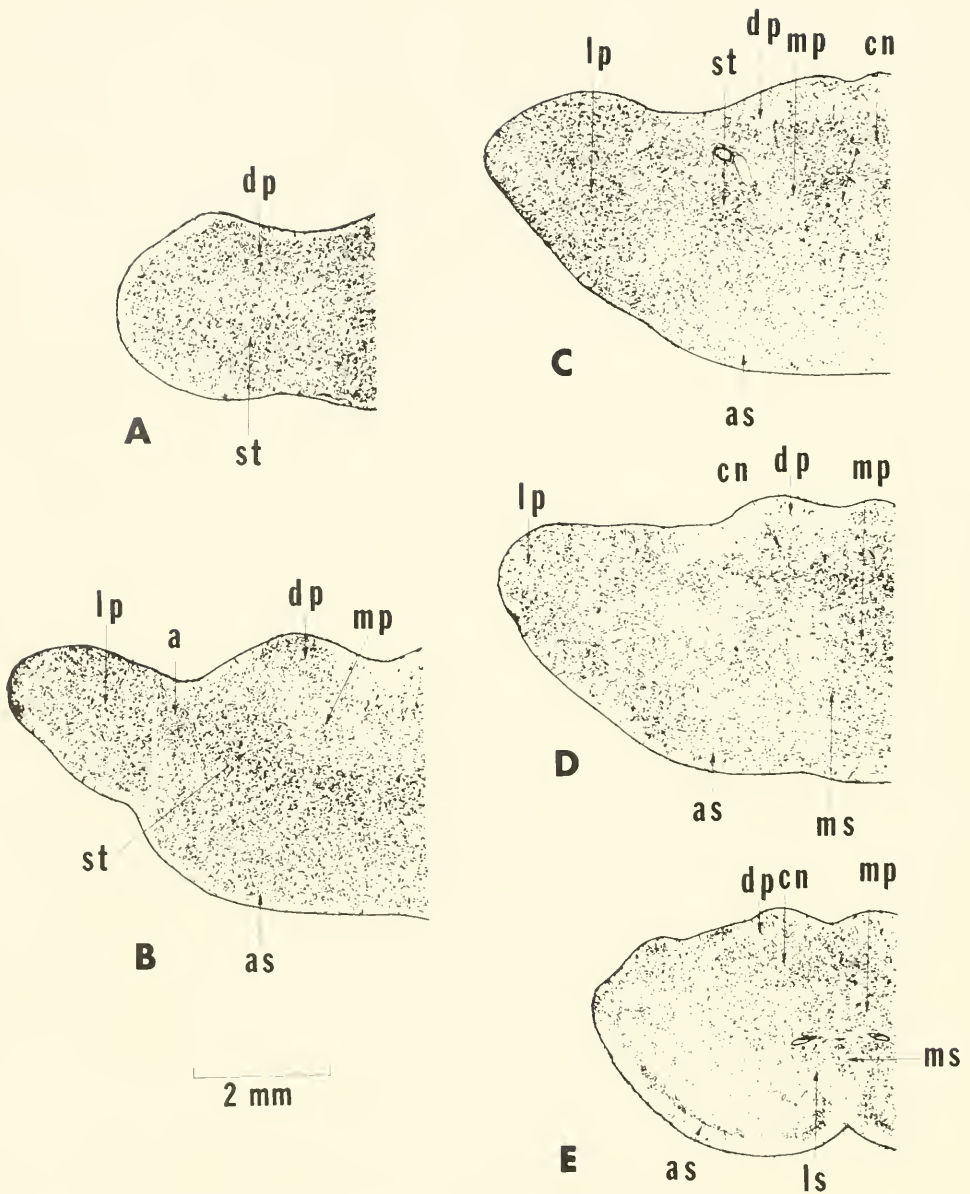
Figure 15   Transverse sections through the right telencephalic hemisphere of the clear-nose skate, *Raja eglanteria*. The levels of these sections are indicated in Figure 16B. a, cell group a (possible pallial amygdala); as, area superficialis basalis; cn, central nucleus; dp, dorsal pallium; lp, lateral pallium; ls, lateral septal nucleus; mp, medial pallium; ms, medial septal nucleus; st, striatum.
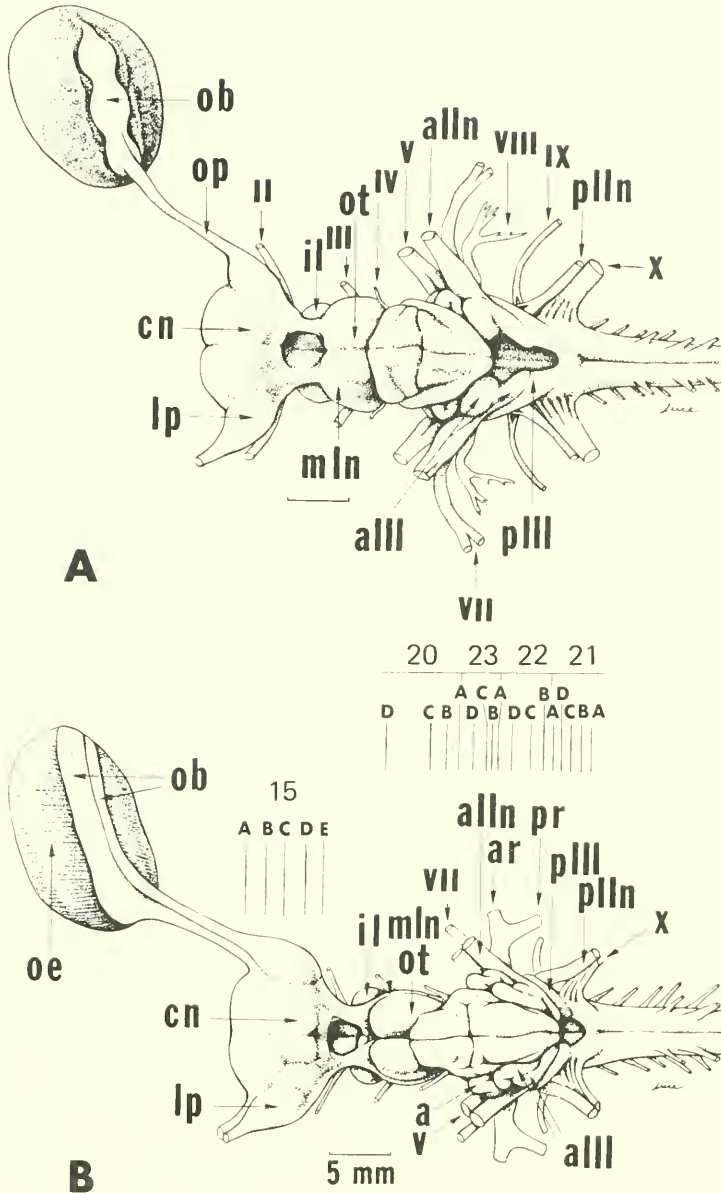
Figure 16 Dorsal view of the brain of *Platyrhinoidis triseriata*, the thornback skate (A) and *Raja eglanteria*, the clearnose skate (B). a, auricle; alll, anterior lateral-line lobe; alln, anterior lateral-line nerve; ar, anterior ramus of statoacoustic nerve; cn, central nucleus; il, inferior lobe of the hypothalamus; lp, lateral pallium; mln, mesencephalic lateralis nucleus; ob, olfactory bulb; oe, olfactory epithelium; op, olfactory peduncle; ot, optic tectum; plll, posterior lateral-line lobe; plln, posterior lateral-line nerve; pr, posterior ramus of statoacoustic nerve; II, optic nerve; III, oculomotor nerve; IV, abducens nerve; V, trigeminal nerve; VII, facial nerve; VIII, statoacoustic nerve; IX, glossopharyngeal nerve; X, vagal nerve.
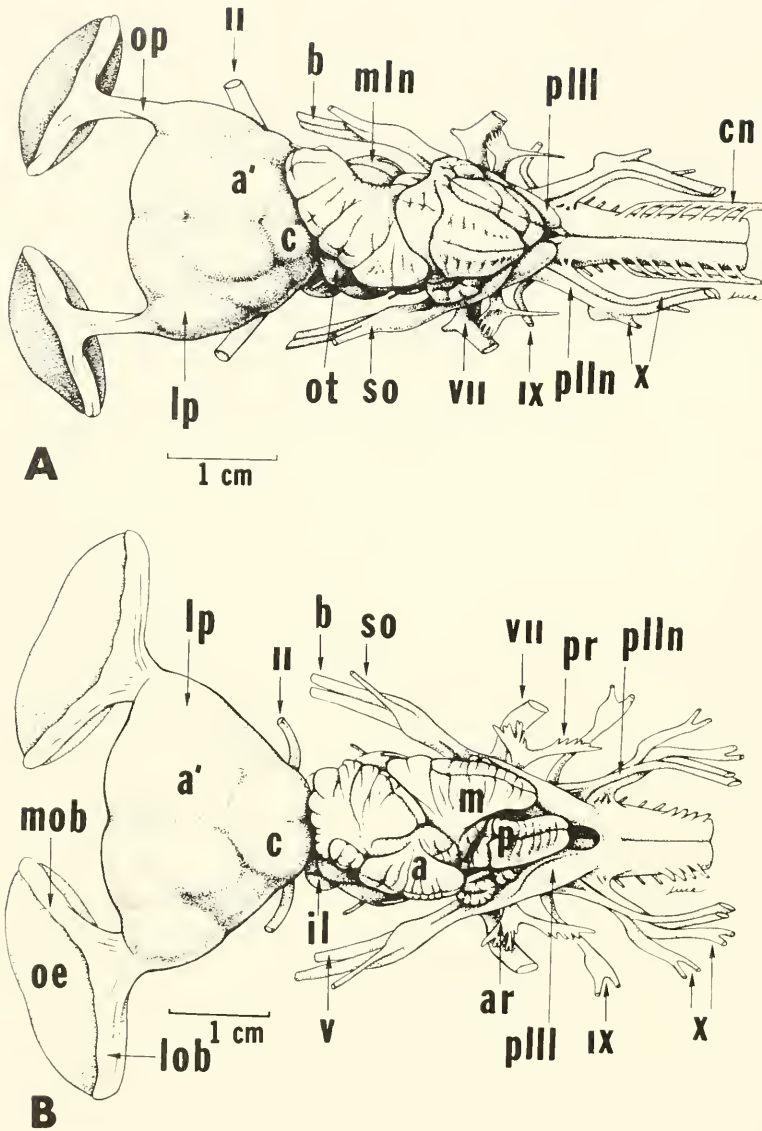
Figure 17   Dorsal view of the brain of *Myliobatis californica*, the bat ray (A) and *Dasyatis americana*, the southern stingray (B). a, anterior lobe of the cerebellum; a', anterior division of the central nucleus of the telencephalon; ar, anterior ramus of the statoacoustic nerve; b, buccal ramus of the anterior lateral-line nerve; c, caudal division of the central nucleus; cn, spinal nerve collector ramus; il, inferior lobe of the hypothalamus; lob, lateral division of the olfactory bulb; lp, lateral pallium; m, middle lobe of the cerebellum; mln, mesencephalic lateralis nucleus; mob, medial division of the olfactory bulb; oe, olfactory epithelium; op, olfactory peduncle; ot, optic tectum; p, posterior lobe of the cerebellum; plll, posterior lateral-line lobe; plln, posterior lateral-line nerve; pr, posterior ramus of the statoacoustic nerve; so, superficial ophthalmic rami of anterior lateral-line and trigeminal nerves; II, optic nerve; V, mandibular and maxillary trunk of the trigeminal nerve; VII, facial nerve; IX, glossopharyngeal nerve; X, vagal nerve.
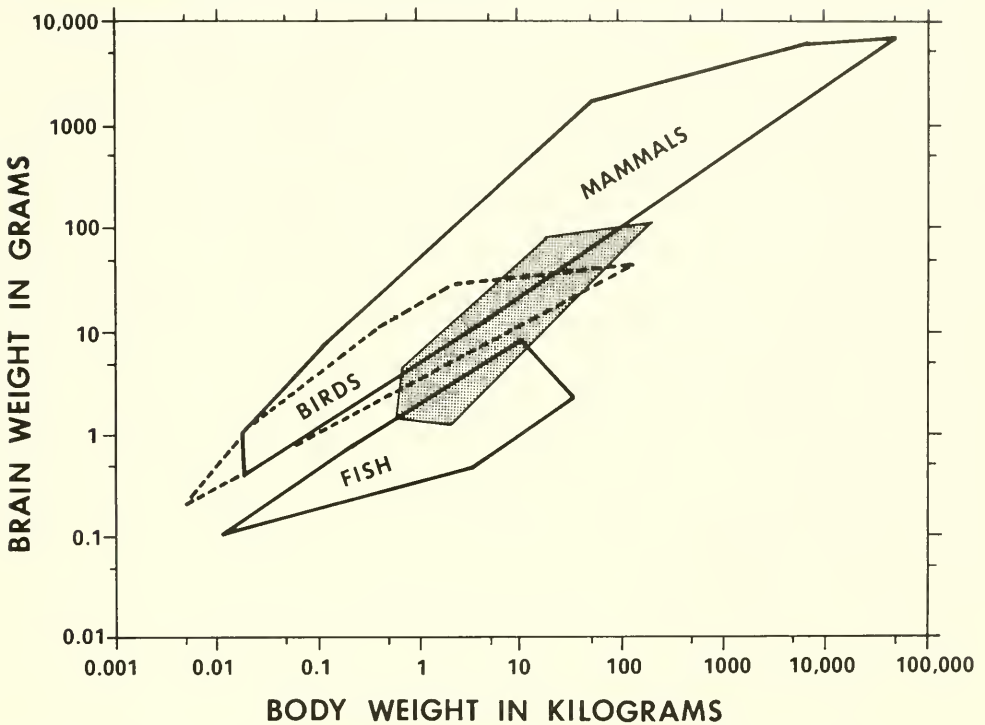
Figure 18   Brain and body weights for four vertebrate classes expressed as minimum convex polygons, after Jerison (1973). Stippled polygon encloses elasmobranch brain-to-body ratios and overlaps polygons for bony fishes, birds, and mammals. (After Northcutt 1977.)

rajiforms are characterized by low brain:body ratios and the more advanced myliobatiforms by the highest brain:body ratios known for elasmobranchs.

Numerical estimates of these brain size differences can be obtained by calculating encephalization quotients (EQ) for the species listed in Table 2. The encephalization quotient is the ratio of actual brain size to expected brain size, defined by the allometric equation for brain:body relations. The expected brain size is an "average" for members of a group and controls for body size. Thus, calculating an EQ allows comparison of different species regardless of body size.

The EQs in Table 2 were calculated by the equation $EQ = E/kp^{\alpha}$, where E and P are brain and body weights, respectively, and k and α are respectively intercept and coefficient of allometry determined from the sample shown in Figure 19. Values of 0.012 (k) and 0.75 (α) for sharks and 0.002 (k) and 1.04 (α) for batoids were used in the calculations, rather than the mean coefficient and intercept for the entire sample. This seemed preferable, as the coefficients of allometry for sharks and batoids are very different; however, it precludes direct comparisons between sharks and batoids, as the batoid EQs would be higher if the calculations were based on a mean α and k.
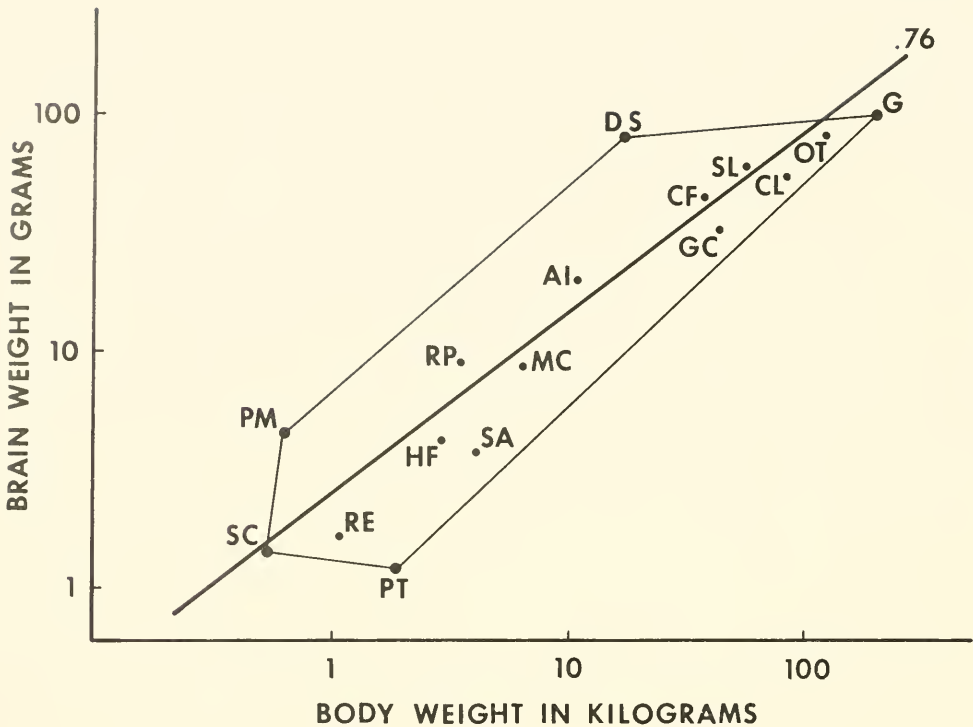
Figure 19 Detailed elasmobranch minimum convex polygon, illustrating positions of various taxa. Interspecific coefficient of allometry is 0.76 with a coefficient of determination of 0.86. AI, *Aprionodon isodon*; CF, *Carcharhinus falciformis*; CL, *Carcharhinus leucas*; DS, *Dasyatis sabina*; G, *Galeocerdo cuvieri*; GC, *Ginglymostoma cirratum*; HF, *Heterodontus francisci*; MC, *Mustelus canis*; OT, *Odontaspis taurus*; PM, *Potamotrygon motoro*; PT, *Platyrhinoidis triseriata*; RE, *Raja eglanteria*; RP, *Rhinobatos productus*; SA, *Squalus acanthias*; SC, *Scyliorhinus caniculus*; SL, *Sphyrna lewini*. (After Northcutt 1977.)

Sharks and batoids listed in Table 2 are ranked in descending order of their *EQ* values. These data suggest that galeomorph sharks possess higher *EQ*s than squalomorph sharks and that myliobatiforms possess *EQ* values 3 to 10 times larger than those of rajiforms. The elasmobranch sample now available is too small to establish meaningful confidence intervals for the *EQ* value of a given species; thus the exact ranking of any species may be relative.

Further study will certainly alter the present boundaries of the chondrichthian polygon. The samples to date are extremely small, and no brain:body data exist for chimaeras or large squalomorph sharks. Data on chimaeras should be particularly interesting, as this group represents a sister radiation of the elasmobranchs, with its own neural specializations. Data on the large squalomorph sharks should extend the lower boundary of the present polygon if the low encephalization quotient for *Squalus* is typical of

the group. Existing data (Bauchot et al., 1976) on the angel shark *Squatina* would change the lower boundary considerably if included.

Data on the advanced batoids (myliobatids, rhinopterids, and mobulids) are particularly needed. Several of the myliobatiforms reach body weights of 50 to 1000 kg, and thus the present polygon is greatly abbreviated. Future considerations should exclude the data of Crile and Quiring (1940) on *Dasyatis sabina*. Two different brain weights have been reported for the same specimen (Crile and Quiring 1940, Quiring 1950) and these values are further complicated by possible misidentification of the specimen. Bigelow and Schroeder (1953) claim that *D. sabina* is the smallest of the North Atlantic stingrays. The largest specimen reported by Bigelow and Schroeder was 39 cm wide thus a weight of 18 kg, as reported by Crile and Quiring, is impossible.

Similarly, the absence of sharks in the upper half of the polygon in Figure 19 may be an error due to small sample size. Data on smaller carcharhinid and sphyrnid species might extend the present distribution; such data are needed before it is concluded that the advanced batoids possess higher brain:body ratios than the sharks.

The common conception that chondrichthians are small-brained creatures is clearly false, but how are the brains of chondrichthians organized? Do these animals possess massive lower brain centers, or do they possess well-developed forebrains like those of birds and mammals? An analysis of the data presented in Figure 1, on the relative development of the major divisions of the brain in a number of chondrichthians, reveals a wide range of variation.

Species such as *Hydrolagus*, *Squalus*, and *Platyrhinoidis* possess relative forebrain development comparable to that of teleosts and amphibians, while the advanced galeomorph sharks possess relative forebrain development comparable to that of endothermic vertebrates (Ebbesson and Northcutt 1976). The distribution of high telencephalic percentages in both sharks and batoids suggests that these levels of neural development have occurred independently and that several levels of development exist within both radiations.

Analysis of brain divisons as percentages of total brain weight or volume indicates which divisions of the brain are highly developed. However, it fails to account for the possibility of independent atrophy or hypertrophy of other brain divisions. Thus, this is not the most accurate assessment of the relative development of brain divisions among various species. A more meaningful numerical analysis is obtained by calculating ratios for brain division weight to body weight. Using a "quick" method (Jerison 1973) to correct for body weight with a coefficient of allometry of 0.76 (α determined from sample presented in Figure 19), I have calculated the relative weights (Table 3) of a sample of brain divisions of the species listed in Table 4. The figures obtained have been multiplied by 10^4 to avoid decimals. This method is "quick" in the sense that it is a first approximation. Given a sufficiently large sample, it would be possible to determine a coefficient of allometry and intercept for each brain division in both sharks and skates. Since all

Table 3. Elasmobranch brain division:body ratios.

Species	Olfactory bulbs	Telencephalon	Diencephalon	Mesencephalon	Cerebellum	Medulla
Squalus acanthias	4	15	4	9	12	17
Mustelus canis	14	31	7	11	16	19
Carcharhinus falciformis	4	81	8	12	14	13
Sphyrna lewini	10	77	6	9	28	18
Platyrhinoidis triseriata	3	10	3	4	5	12
Raja eglanteria	2	25	5	10	13	22
Rhinobatos productus	9	64	14	18	26	50
Myliobatis freminvillii	23	79	10	18	41	34
Dasyatis centroura	21	127	14	23	48	39

brain divisions do not change at the same rate, the coefficients would differ for different brain divisions (Northcutt et al. 1978). Coefficients for each brain division would provide a more accurate measure of differences in brain development among different species. However, available data are insufficient for these calculations, and the mean coefficient of allometry for elasmobranchs was used instead.

When corrections are made for body size, the telencephalon shows a progressive increase in size from *Squalus* to *Sphyrna* and *Carcharhinus*. A similar trend is seen in the batoids from *Platyrhinoidis* to *Dasyatis*, which exhibits the most developed telencephalon of the elasmobranchs examined (a 13-fold increase over *Platyrhinoidis*). Similar increases characterize the other brain divisions in these groups (Table 3), with marked exceptions such as the large olfactory bulbs of *Mustelus* and the medulla of *Rhinobatos*. In general, however, *Sphyrna* (among sharks) and *Dasyatis* (among batoids) reveal increased size in most brain divisions.

Sphyrna exhibits the most complex cerebellar foliation and the largest cerebellar increase (fourfold) among sharks. Among the batoids, *Dasyatis* exhibits a tenfold increase in cerebellar size over *Platyrhinoidis*. The data also suggest that cerebellar foliation is not merely a simple response to increase in cortical volume. *Mustelus* exhibits very distinct cerebellar convolutions (Figures 12, 14), and its cerebellar volume is only 33% larger than that of *Squalus* while cerebellar volume increases 260% from *Platyrhinoidis* to *Raja*, with almost no change in cerebellar foliation (Figure 16).

Finally, although encephalization quotients exhibit approximately the same range in sharks and batoids (a fivefold to sixfold increase in both groups), the batoids exhibit twice as much actual variation among all brain divisions as the sharks. This is particularly true for mesencephalic and medullar variation, though, again, this may be a function of small sample size and must be determined by further study.

Central Nervous System Organization

In chondrichthians and other vertebrates, the greatest evolutionary changes in the brain occur in the roofs of the forebrain, midbrain, and hindbrain. The

Table 4.   Elasmobranch brain division weights.

Species	Body weight (kg)	Brain division weight (g)					
		Olfactory bulbs	Telen-cephalon	Dien-cepahlon	Mesen-cephalon	Cere-bellum	Medulla
Squalus acanthias	4.2	0.2129	0.8337	0.2532	0.5353	0.7016	0.9813
Mustelus canis	6.5	1.1172	2.4818	0.5757	0.8661	1.2600	1.5404
Carcharhinus falciformis	36.3	1.1248	23.7518	2.2958	3.3577	4.1974	3.8909
Sphyrna lewini	55.7	4.1916	31.1376	2.3952	3.5928	11.3772	7.1856
Platyrhinoidis triseriata	2.1	0.0920	0.3253	0.0991	0.1365	0.1656	0.4088
Raja eglanteria	1.1	0.0478	0.5049	0.1087	0.2081	0.2727	0.4406
Rhinobatos productus	3.6	0.4372	3.2067	0.7105	0.8927	1.3027	2.5325
Myliobatis freminvillii	5.4	1.5834	5.4165	0.7170	1.2651	2.7961	2.3235
Dasyatis centroura	5.6	1.4410	8.9631	1.0163	1.6301	3.3882	2.7374

floor of the hindbrain (medulla) and midbrain (tegmentum) is much more conservative, and homologous areas are more easily recognized among vertebrates than are areas of the forebrain. The anatomy of each brain region will be summarized, its known connections reviewed, and the variation among chondrichthians noted.

Brain Stem—The brain stem is formed by the floor of the hindbrain and midbrain, excluding the cerebellum and optic tectum. The sensory and motor columns of the spinal cord continue into the brain stem and terminate at its rostral border, marked by the nucleus interstitialis and the oculomotor nucleus (Figures 5, 20). Thus, the brain stem, like the spinal cord, consists of dorsal sensory nuclei and ventral motor nuclei, as well as pathways passing to and from the spinal cord.

Recently, Smeets and Nieuwenhuys (1976) described the brain stems of the sharks *Squalus* and *Scyliorhinus* and reviewed the earlier literature. A similar treatment of batoids does not exist. Thus, I will deal primarily with the brain stem of *Raja*, rather than merely repeating details provided by Smeets and Nieuwenhuys. The nomenclature used for *Raja* is essentially that of Smeets and Nieuwenhuys, and differences between the brain stem of *Raja* and those of other chondrichthians will be noted.

The nuclei of the brain stem will be described under the following headings: (1) somatic motor nuclei, (2) visceral motor nuclei, (3) reticular formation, (4) visceral sensory nuclei, (5) somatic sensory nuclei, and (6) acousticolateralis nuclei.

Somatic motor nuclei—These nuclei comprise a rostral extension of the spinal somatic motor column and consist of a caudal group (Figure ·21A, B) innervating the hypobranchial muscles and a rostral group innervating the extrinsic eye muscles: abducens, trochlear, and oculomotor nuclei. The abducens nucleus (not pictured) consists of a loosely scattered group of medium-size cells extending dorsally from the ventral exit of the abducens nerve roots. This nucleus is located in the brain stem, between the levels indicated in Figure 22B, C.

The trochlear and oculomotor nuclei are well developed and form a continuous column of cells with no distinct break between the two nuclear groups (Figure 20A, B, C). *Raja*, like the lampreys, possesses distinct dorsal and ventral divisions of the oculomotor nucleus (Figure 20C). The dorsal division lies just lateral and ventral to the medial longitudinal fascisulus, as in most vertebrates, while the ventral division forms a second distinct nucleus of large fusiform and triangular cells located among the exiting roots of the oculomotor nerve. This peculiar oculomotor pattern has been experimentally confirmed in *Dasyatis* by Rosiles et al. (1977).

All of the somatic motor nuclei lie in close proximity to the medial longitudinal fasciculus (mlf, Figures 20–23). The medial longitudinal fasciculus can be traced rostrally to the level of nucleus interstitialis (Figure 5) and caudally into the spinal cord. The axons of this pathway both ascend
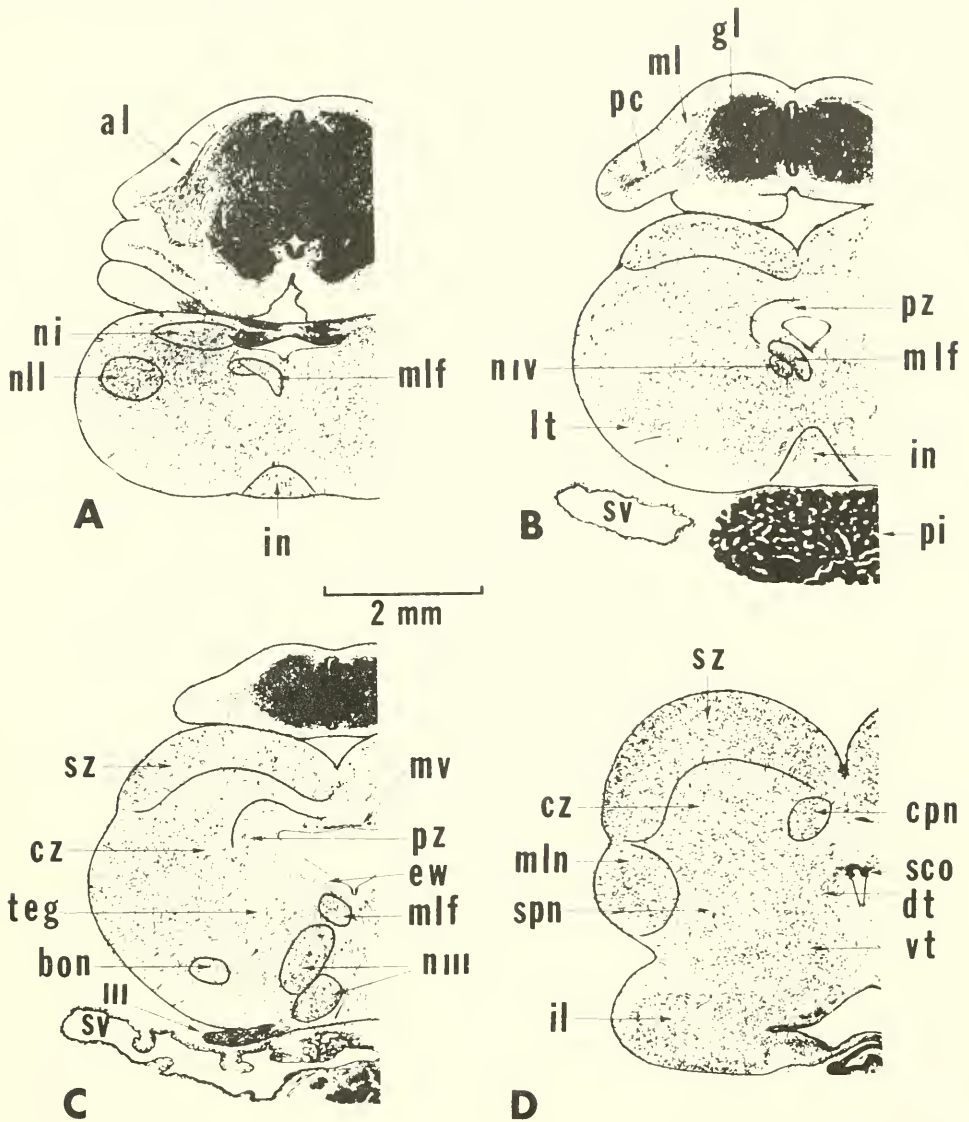
Figure 20 Transverse sections through the mesencephalon and caudal diencephalon of *Raja eglanteria*. Magnification of d is 0.2X greater than other sections. al, anterior lobe of the cerebellum; bon, basal optic nucleus; cpn, central pretectal nucleus; cz, central tectal zone; dt, dorsal thalamus; ew, Edinger-Westphal nucleus; gl, granular layer of cerebellar cortex; il, inferior lobe of hypothalamus; in, interpeduncular nucleus; lt, lateral tegmental nucleus; ml, molecular layer of cerebellar cortex; mlf, medial longitudinal fasciculus; mln, mesencephalic lateralis nucleus; mV, trigeminal mesencephalic nucleus; ni, nucleus isthmi; nll, nucleus of the lateral lemniscus; nIII, oculomotor nucleus; nIV, trochlear nucleus; pc, Purkinje cell layer of the cerebellar cortex; pi, pituitary gland; pz, periventricular or deep tectal zone; sco, subcommissural organ; spn, superficial pretectal nucleus; sv, saccus vasculosus; sz, superficial tectal zone; teg, midbrain tegmentum; vt, ventral thalamus; III, oculomotor nerve.
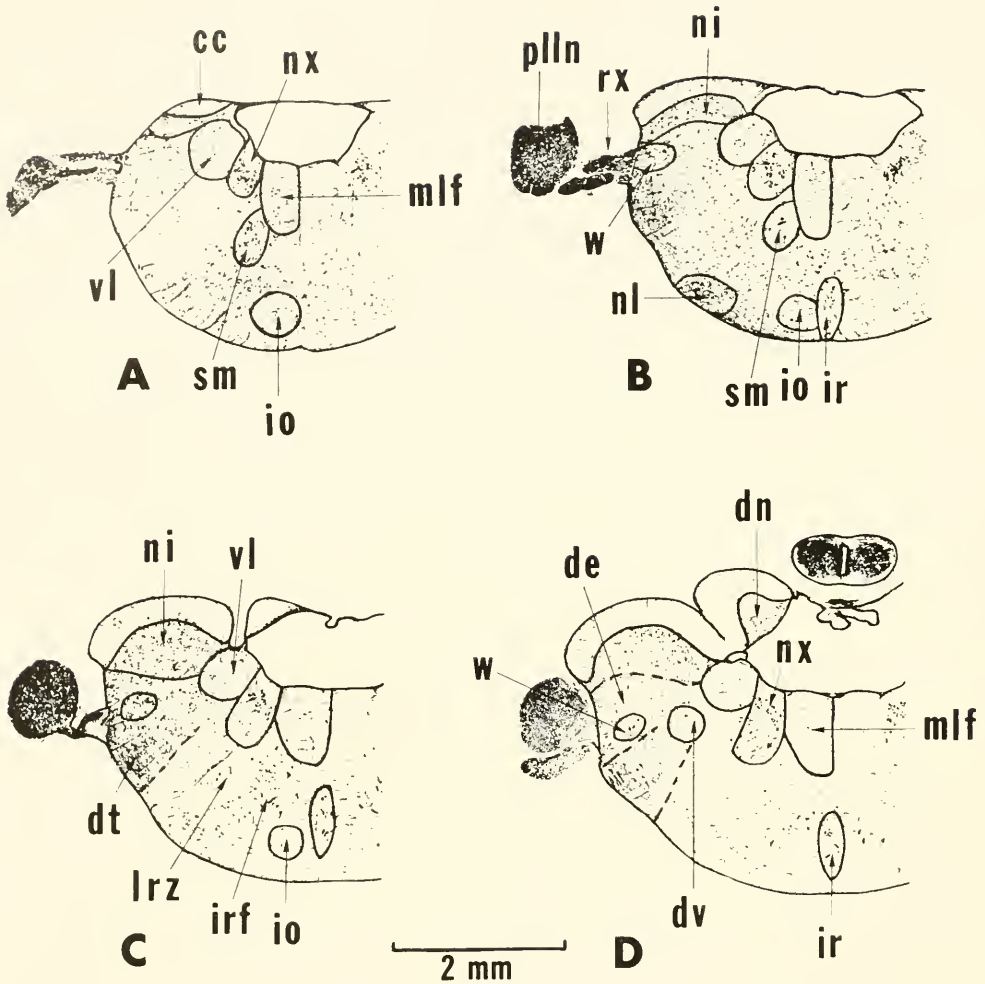
Figure 21   Transverse sections through the caudal hindbrain of the clearnose skate, *Raja eglanteria*. The levels of these sections and those illustrated in Figures 20, 22, and 23 are indicated in Figure 16B. cc, cerebellar crest; de, descending tract and nucleus of VIII; dn, dorsal nucleus of the anterior lateral line lobe; dt, descending trigeminal tract; dv, descending trigeminal nucleus; io, inferior olive; ir, inferior raphe nucleus; irf, inferior reticular formation; lrz, lateral reticular zone; mlf, medial longitudinal fasciculus; ni, nucles intermedius of the posterior lateral-line lobe; nl, nucleus of the lateral funiculus; nx, vagal motor nucleus; plln, posterior lateral-line nerve; rx, roots of the vagal nerve; sm, spinal motor column (hypoglossal nucleus); vl, vagal lobe (nucleus solitarius); w, nucleus w (possible lateral vagal motor nucleus or primary sensory neurons).

and descend throughout the brain stem and arise from a number of different sources. All existing vertebrate experimental data indicate that the vestibular nuclei form extensive connections with the extrinsic eye muscle nuclei via the medial longitudinal fasciculus. Experimental evidence for *Dasyatis* indicates that the axons of nucleus interstitialis extend from midbrain levels into the spinal cord (R. B. Leonard, personal communication).
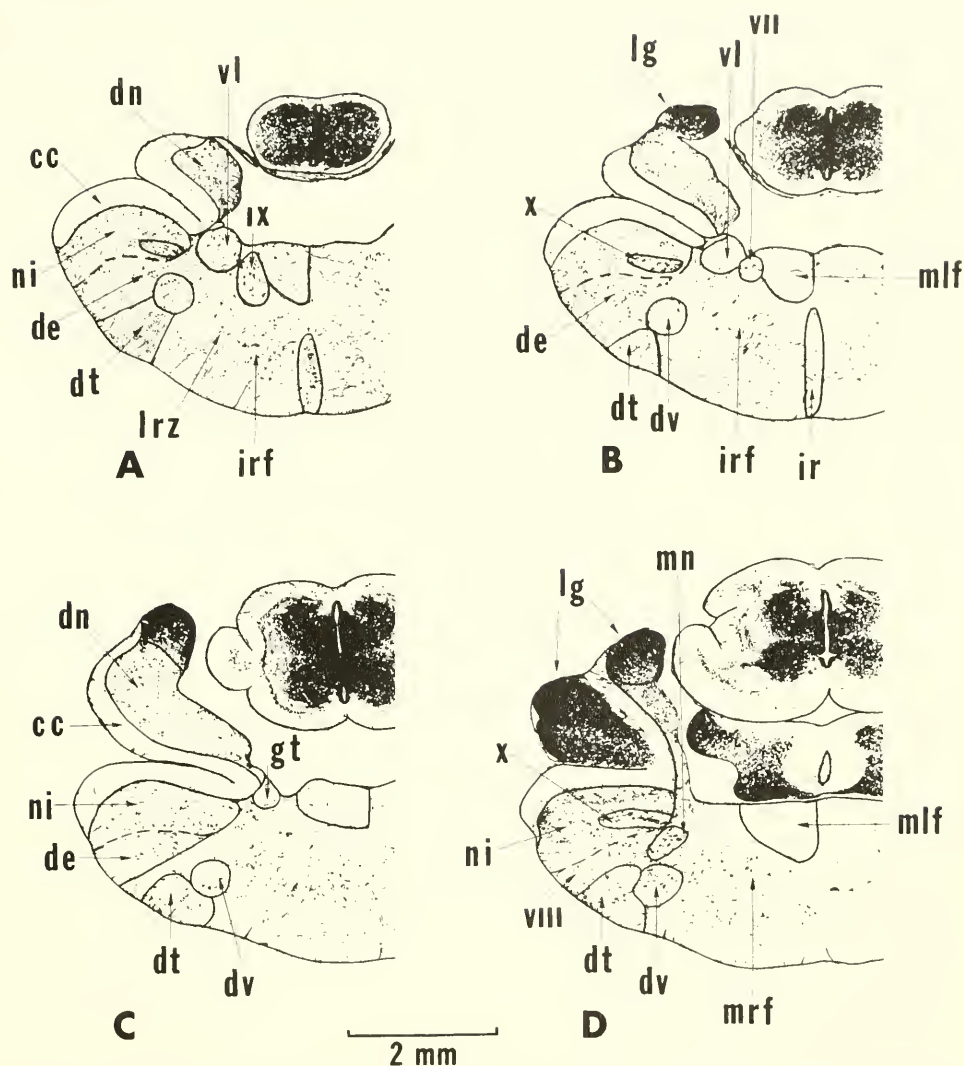
Figure 22 Transverse sections through middle levels of the hindbrain of *Raja eglanteria*. cc, cerebellar crest; de, descending tract and nucleus of VIII; dn, dorsal nucleus of the anterior lateral-line lobe; dt, descending trigeminal tract; dv, descending trigeminal nucleus; gt, secondary gustatory tract; ir, inferior raphe nucleus; irf, inferior reticular formation; lg, lateral granular layer; lrz, lateral reticular zone; mlf, medial longitudinal fasciculus; mn, magnocellular nucleus of VIII; mrf, medial reticular formation; ni, nucleus intermedius of the posterior lateral-line lobe; vl, vagal lobe; VII, facial motor nucleus; VIII, roots of statoacoustic nerve; IX, glossopharyngeal motor nucleus; X, nucleus X (possible cells of origin of efferent fibers to VIII and lateralis nerves).
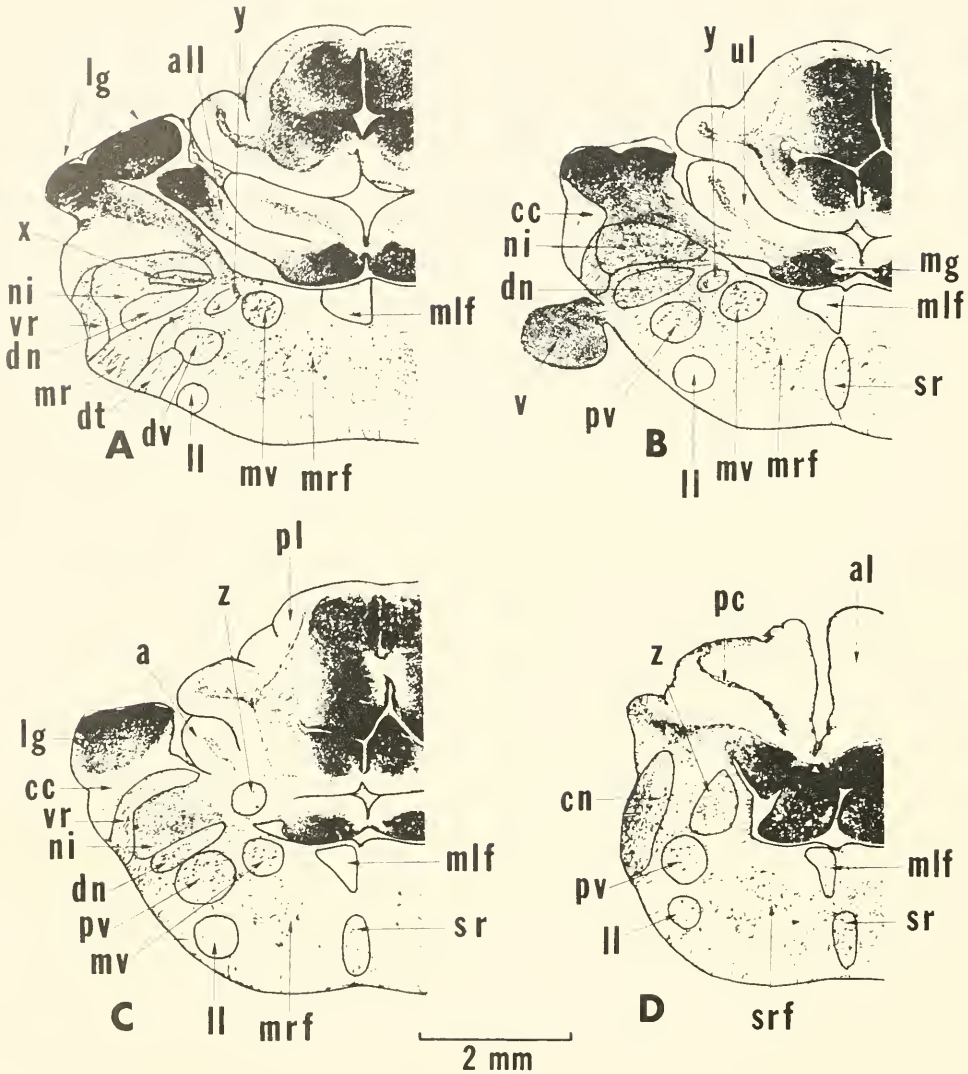
Figure 23   Transverse sections through the rostral hindbrain of *Raja eglanteria*. a, auricle; al, anterior lobe of the cerebellum; all, lower leaf of the auricle; cc, cerebellar crest; cn, cerebellar nucleus; dn, dorsal nucleus of VIII; dt, descending trigeminal tract; dv, descending trigeminal nucleus; lg, lateral granular layer; ll, lateral lemniscus; mg, medial granular layer; mlf, medial longitudinal fasciculus; mr, trigeminal root; mrf, medial reticular formation; mv, trigeminal motor nucleus; ni, nucleus intermedius of the posterior lateral line lobe; pc, Purkinje cell layer; pl, posterior lobe of the cerebellum; pv, principal trigeminal sensory nucleus; sr, superior raphe nucleus; srf, superior reticular formation; ul, upper leaf of auricle; V, trigeminal root; vr, ventral ramus of anterior lateral-line nerve; X, nucleus X of posterior lateral-line lobe; y, nucleus y (possible locus coerulus or primary sensory neurons); Z, nucleus z (possible medial cerebellar nucleus or secondary gustatory nucleus).

Visceral motor nuclei—The motor nuclei of X, IX, VII, and V with the Edinger-Westphal nucleus form a ventrolateral column of motor neurons (Figures 20–23) throughout the brain stem. The motor nuclei of X, IX, and VII consist of a single continuous column of large neurons in the caudal medulla (Figures 21, 22B), while the motor nucleus of V (Figure 23A, B, C) is located more rostrally as a separate group.

Rosiles et al. (1977) confirmed experimentally that the motor nuclei of X, IX, and VII do arise from a single caudal brain stem group in *Dasyatis*. There is some variation in the organization of the caudal visceral motor nuclei in chondrichthians. In chimaeras, the vagal portion of the motor column can be divided into separate rostral and caudal divisions (Addens 1933). Addens suggested that the rostral division might be branchiomotor and the caudal division splanchnic motor—homologous in land vertebrates to the nucleus ambiguus and the dorsal motor nucleus of the vagus, respectively.

In *Squalus*, Smeets and Nieuwenhuys recognized a lateral motor nucleus of the vagus, in addition to the usual more medial motor nucleus recognized in fishes. A similar condition may exist in *Raja*, as a distinct group of large neurons is seen among the roots of the vagus (Figure 21) and is termed nucleus w. These cells in *Raja* may constitute a lateral motor nucleus of the vagus, or primary sensory neurons similar to the Rohon-Beard cells of the spinal cord and mesencephalic trigeminal nucleus. A similar cell group is lateral to the motor nucleus of V in *Raja* and is identified as nucleus y (Figure 23A, B). The cells of this nucleus might be caudally situated neurons of the mesencephalic nucleus of V, but they do not look like the more rostrally located mesencephalic V neurons. Nucleus y might also be a lateral division of the motor nucleus of V, or, possibly, a homologue of the locus coeruleus of land vertebrates.

In *Squalus*, Smeets and Nieuwenhuys (1976) also recognized an additional nucleus, rostral to the motor nucleus of VII, that may be visceral motor in nature (their nucleus A). They did not recognize a similar group in *Scyliorhinus*, nor have I been able to recognize such a nucleus in *Raja*.

The most rostral nucleus of the visceral motor column is the midbrain nucleus of Edinger-Westphal, situated dorsolateral to the dorsal division of the oculomotor nucleus (Figure 20C). This nucleus consists of small neurons and probably gives rise to the autonomic preganglionic axons innervating the dilatator iridis muscle of the eye.

Reticular formation—The reticular formation extends the length of the hindbrain and consists of three longitudinal zones: a median or midline zone, a medial large-celled reticular zone, and a lateral small-celled reticular zone.

In *Raja*, the median or midline reticular zone consists of inferior and superior raphe nuclei (Figures 21B, 22B, 23B, C, D) composed of medium to large neurons whose cell bodies straddle the midline with dendrites extending into both halves of the medulla. A superior raphe nucleus was identified in *Squalus* and *Scyliorhinus* by Smeets and Nieuwenhuys (1976). However,

they did not recognize a distinct inferior raphe nucleus. No experimental data exist for the raphe nuclei of chondrichthians, but these nuclei in other vertebrates are characterized by marked 5-hydroxytryptamine (5-HT) activity, and they form extensive connections with both spinal cord and forebrain nuclei.

The medial reticular zone consists of both medium and large polygonal neurons and occupies a ventrolateral position beneath the medial longitudinal fasciculus (Figures 21-23). The smaller cells form a more or less continuous column along the entire length of the hindbrain, but the large neurons are discontinuous and can be divided into inferior, medial, and superior large-celled components. These divisions were first recognized by van Hoëvell (1911) in *Raja* and have been since confirmed by Smeets and Nieuwenhuys (1976) in *Squalus* and *Scyliorhinus*. The inferior large-celled reticular nucleus (inf. Figures 21C, 22B) occupies the caudal medulla, adjacent to the caudal visceral motor column, and is replaced more rostrally by the medial large-celled reticular nucleus (mrf, Figures 22D, 23C). The medial nucleus occupies the medullar region marked by the entry of the statoacoustic and trigeminal nerves, and is replaced rostrally by the superior large-celled reticular nucleus (srf, Figure 23D), which extends through the rostral medulla into the caudal midbrain isthmic region.

The lateral reticular zone consists of small neurons located between the large-celled medial reticular zone and the descending tract of V (lrz, Figures 21–23), and it is likely homologous to the parvicellular reticular nucleus of mammals. The lateral reticular zone is the terminal site for most of the ascending spinal pathways forming the spinal lemniscus (Hayle 1973a) and is also the target of an ipsilateral descending cerebello-bulbar pathway (Ebbesson and Campbell 1973). The neurons of the large-celled medial reticular zone have long, branching dendrites that extend into the lateral reticular zone. Thus, in all likelihood, they also contact fibers of the ascending spinal lemniscus.

No experimental studies exist for the reticular formation of chondrichthians, but in other vertebrates the reticular formation is known to form extensive ascending and descending connections with the spinal cord and higher neural centers. The inferior medullar reticular region consists of neuronal populations that give rise to a ventral noradrenergic pathway projecting to the midbrain roof, thalamus, hypothalamus, and basal regions of the telencephalon. In addition, the large-celled reticular zone is the primary target of telencephalic, tectal, and cerebellar efferents linking the brain to the spinal cord by descending reticular pathways. This pattern of connectivity places the reticular formation in an ideal position to function as a pattern generator. The reticular formation consists of both ipsilateral and contralateral pathways projecting to most parts of the spinal cord. Such reticular neurons, projecting over wide segments of the cord and motor nuclei of the medulla, could produce a wide range of output signals depending on the temporal and spatial summation of arriving afferents from very diverse centers of the spinal cord, cerebellum, tectum, hypothalamus, and telencephalon.

Two additional groups of cells, the inferior olive (io, Figure 21A, B, C) and the nucleus of the lateral funiculus (ne, Figure 21B), are closely associated topographically with the reticular formation and are probably phylogenetically derived from this formation. The inferior olivary nucleus consists of small, spherical cells embedded in a densely staining neuropil. The efferents of this cell group in chondrichthians are unknown, but a similar nucleus in bony fishes projects to the corpus of the cerebellum (unpublished observations) and is probably homologous to the accessory olivary nuclei of mammals.

The nucleus of the lateral funiculus consists of small- to medium-sized fusiform or bipolar neurons scattered among the fibers at the outer edge of the ascending lateral or spinal lemniscus. The efferents of this nucleus are unknown in cartilaginous fishes, but in mammals a similarly situated nucleus projects to the cerebellar vermis.

Visceral sensory nuclei—The visceral sensory fibers of VII, IX, and X—carrying gustatory, pain, temperature, pressure, and respiratory chemoreceptor information—terminate in a longitudinal column of cells dorsolateral to the visceral motor column, termed the vagal lobe (vl, Figures 21, 22B). The lobe consists of small- to medium-sized, spherical or fusiform neurons scattered among fascicles of the visceral sensory fibers. Rostrally the vagal lobe gives rise to a well-encapsulated bundle, the secondary gustatory tract (gt, Figure 22C), which ascends rostrally to a position medial to the nucleus intermedius of the posterior lateral line lobe, where it can no longer be recognized as a distinct bundle. No details about the higher order organization of the visceral sensory systems in chondrichthians are available, but in bony fishes the vagal lobe projects, via the secondary gustatory tract, to a distinct secondary gustatory nucleus located ventral to the cerebellar corpus and nuclei. Medial to the cerebellar nucleus, a distinct nucleus z (Figure 23D) may represent a secondary gustatory nucleus in *Raja*, but experimental study is needed to test this possibility. In other fishes, the secondary gustatory nucleus projects to the caudal inferior lobe of the hypothalamus (Finger 1976). Whether or not gustatory information reaches telencephalic levels in fishes is unknown.

Somatic sensory nuclei—The dorsal column nuclei, the nucleus of the descending tract of V, the principal trigeminal nucleus, and the trigeminal mesencephalic nucleus constitute the somatic sensory systems of the brain stem. In land vertebrates, the dorsal column nuclei (nuclei cuneatus and gracilis of mammals) are located in the dorsal obex region of the medulla. They receive afferents via the dorsal funiculus of the spinal cord, and their axons give rise to the medial lemniscus after crossing the medullar midline as the internal arcuate fibers. According to Hayle (1973a), the dorsal funiculus of *Scyliorhinus* does not possess long ascending fibers, nor were Smeets and Nieuwenhuys (1976) able to recognize distinct dorsal column nuclei in *Scyliorhinus* or *Squalus*. However, Ebbesson (1972) traced dorsal funicular fibers to an obex cell group he termed the dorsal column nuclei, following

hemicordotomy at the second spinal segment in the nurse shark, *Ginglymos-toma*.

Distinct dorsal column nuclei were not identified in *Raja*, and further experimental studies are needed to assess the development of the medial lemniscal system in cartilaginous fishes.

The entering sensory fibers of the trigeminal nerve form ascending and descending tracts in *Raja*. The ascending fibers terminate in the principal sensory trigeminal nucleus (pv, Figure 23B, C, D) located in the rostral medulla. The principal nucleus consists of medium-sized polygonal cells embedded in a dense neuropil. Smeets and Nieuwenhuys (1976) were unable to recognize a principal nucleus in *Scyliorhinus*, and tentatively identified such a nucleus in *Squalus* as nucleus C.

In mammals the principal nucleus projects to the thalamus, which in turn projects to primary sensory cortex. In birds and reptiles the principal nucleus projects directly to a rostral basal nucleus of the telencephalon, which in turn projects to the more dorsally located pallium. No specific experimental data exist for the central projections of the trigeminal nerve in cartilaginous fishes. However, Schroeder and Ebbesson (1974) observed a bilateral ascending pathway to a medial subpallial area immediately dorsal to area superficialis basalis following mesencephalic lesions in nurse sharks. This pathway may represent a trigemino-telecephalic pathway as in birds and reptiles.

The descending fibers of the trigeminal nerve form a distinct descending trigeminal tract (dt, Figures 21–23), which is traced caudally to the obex region of the medulla. Medium-sized neurons are scattered in the dorsomedial angle of the descending tract, throughout its rostrocaudal extent, and constitute a nucleus of the descending trigeminal tract (dv, Figures 21, 22, 23A). In other vertebrates, somatic sensory fibers of the VIIth, IXth and Xth cranial nerves also contribute to the descending trigeminal tract and nucleus, and thus constitute a major system relaying somatic pain and temperature information to the forebrain. Again, comparable information about chondrichthians is wanting.

The trigeminal mesencephalic nucleus consists of large primary sensory neurons whose cell bodies are in the periventricular zone of the optic tectum (mV, Figure 20C). Witkovsky and Roberts (1975) analyzed the mesencephalic trigeminal nucleus in *Scyliorhinus*, *Mustelus*, and *Raja*, concluding that the nucleus consists of at least two populations of cells. The peripheral neurite of a rostral population was observed entering the trigeminal nerve innervating the teeth and adjacent skin of the jaws. These cells are also said to possess a collateral process ending on the neurons of the trigeminal motor nucleus and forming a monosynaptic reflex mediating jaw closure (Roberts and Witkovsky 1975). A second, more caudal population (approximately 15% of the mesencephalic "trigeminal" neurons) was not observed to send a neurite into the trigeminal nerve but was seen to project caudally and medially to terminate among the Purkinje cells of the anterior lobe of the cerebellum. Roberts' and Witkovsky's studies suggest the existence of two distinct

populations of mesencephalic cells: a large group. functioning as a sensory neuron in a monosynaptic jaw adduction reflex; and a smaller group, an interneuron-cerebellar pathway of unknown function.

Acousticolateralis nuclei—The acousticolateralis nuclei and their associated nerves form a prominent complex in the rostral hindbrain. This complex is frequently termed the acousticolateralis area, or, in the older literature, the acousticolateralis tubercle (Kappers et al. 1936). An understanding of the complex relationships of this area requires examination of the cranial nerves associated with the acousticolateralis area. Three cranial nerves terminate in the various nuclei forming the acousticolateralis area: anterior and posterior lateral line nerves and a statoacoustic nerve (Figures 8, 9, 12, 13, 16, 17). The anterior and posterior lateral-line nerves innervate the lateral line and associated sensory organs of the head and trunk, respectively.

The lateralis nerves are not numbered because until recently they were believed to be components of the facial and vagal nerves (Kappers et al. 1936). However, embryological evidence has demonstrated that the lateralis nerves arise from a dorsolateral series of head placodes, as does the statoacoustic nerve, and that together these nerves form a series that parallels the branchiomeric nerves, rather than comprising components of the branchiomeric series. This new view is strongly reinforced by the facts that the lateralis nerves possess separate and distinct ganglia (of different embryonic origin than those of the branchiomeric nerves) and that they enter the medulla separately and terminate in sensory nuclei distinct from any of the branchiomeric nerves (McCready and Boord 1976, Boord and Campbell 1977).

The anterior lateral line nerve innervates the mechanoreceptors (neuromasts) of the ordinary lateral line, as well as the electroreceptors located on the head and termed ampullae of Lorenzini. The peripheral distribution and central roots of the anterior lateral line are summarized in Figures 12, 24, 25. The anterior lateral-line nerve consists of four peripheral branches: superficial ophthalmic, buccal, otic, and external mandibular. These rami are closely associated peripherally with rami of cranial nerves V, VII, and VIII but do not anastomose with these rami, and each lateralis ramus possesses its own distinct ganglion (McCready and Boord 1976). As each peripheral lateralis branch or ramus approaches the medulla it divides into dorsal and ventral roots. The dorsal root carries only ampullary fibers, and the ventral root only mechanoreceptor or neuromast fibers. The dorsal root enters the anterior lateral-line lobe (Figures 16, 25, 26), and the ventral root enters the posterior lateral-line lobe (Boord and Campbell 1977).

The posterior lateral-line nerve innervates the trunk neuromasts and consists of a single root as it approaches the medulla and enters only the posterior lateral-line lobe. Thus the anterior lateral-line lobe receives electroreceptive information only via the ampullary system, and the posterior lateral line lobe receives mechanoreceptive information from the head and trunk neuromasts (Boord and Campbell 1977).

Figure 24 (A) Topographic relationships of the various rami of the anterior lateral-line nerve to the Vth, VIIth and VIIIth cranial nerves in *Mustelus canis* from a dorsolateral view of the brain stem. (B) Buccal and otic rami of the anterior lateral-line nerve are removed to show the course of the dorsal and ventral roots of the superficial ophthalmic and external mandibular branches. Circles indicate positions of the ganglia. ALL, anterior lateral-line lobe; b, buccal ramus of anterior lateral-line nerve; Gb, ganglion of the buccal ramus; Gg, geniculate ganglion; Gme, ganglion of the external mandibular ramus of the anterior lateral-line nerve; God, ganglion of dorsal root of otic ramus of the anterior lateral-line nerve; Gos, ganglion of superficial ophthalmic ramus of anterior lateral-line nerve; Gov, ganglion of the ventral root of the otic ramus of the anterior lateral-line nerve; h, hyomandibular trunk; NLLp, posterior lateral-line nerve; o, otic ramus of the anterior lateral-line nerve; os, superficial ophthalmic ramus of anterior lateral-line nerve; Rmed, dorsal root of external mandibular ramus of anterior lateral-line nerve; Rmev, ventral ramus of external mandibular ramus of anterior lateral-line nerve; Rosd, dorsal root of superficial ophthalmic ramus of anterior lateral-line nerve; Rosv, ventral root of superficial ophthalmic ramus of anterior lateral-line nerve; Rov, ventral root of otic ramus of anterior lateral-line nerve; Vm, mandicular ramus of V; Vmx, maxillary ramus of V; Vop, deep ophthalmic ramus of V; Vos, superficial ophthalmic ramus of V; RVIIvs, visceral sensory root of VII; VIIvs, visceral sensory ramus of VII; VIII, statoacoustic nerve; IX, glossopharyngeal nerve; X, vagal nerve. (After McCready and Boord, 1976.)
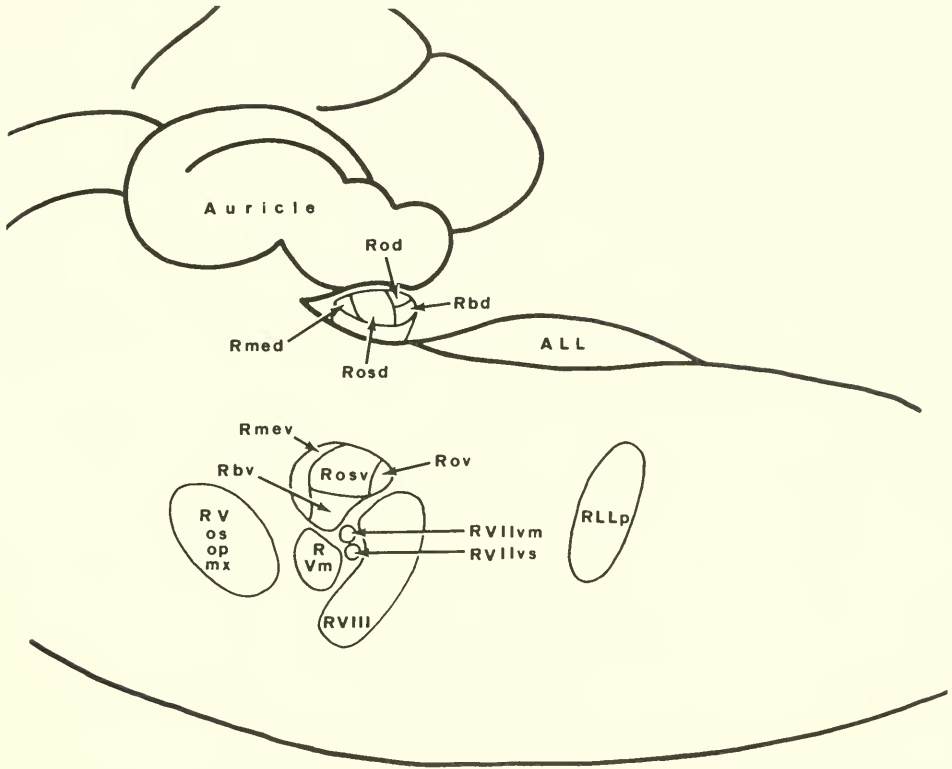
Figure 25   Lateral view of the brain stem of *Mustelus canis* showing the relationships of the various branches of the anterior lateral-line nerve and its dorsal and ventral roots. The superficial roots of Vth, VIIth, VIIIth, and posterior lateral-line nerves are also shown. ALL, anterior lateral-line lobe; Rbd, dorsal root of buccal ramus of anterior lateral-line nerve; Rbv, ventral root of buccal ramus of anterior lateral-line nerve; RLLp, root of posterior lateral-line nerve; Rmed, dorsal root of external mandibular ramus of anterior lateral-line nerve; Rmev, ventral root of external mandibular ramus of anterior lateral-line nerve; Rod, dorsal root of otic ramus of anterior lateral-line nerve; Rosd, dorsal root of superficial ophthalmic ramus of anterior lateral-line nerve; Rosv, ventral root of superficial ophthalmic ramus of anterior lateral-line nerve; Rov, ventral root of otic ramus of anterior lateral-line nerve; RVm, root of mandibular ramus of V; RVos, op, mx, superficial ophthalmic, deep ophthalmic and maxillary roots of V; RVIIvm, visceral motor root of VII; RVIIvs, vsiceral sensory root of VII; RVIII, root of VIII. (After McCready and Boord 1976.)

The statoacoustic nerve consists of anterior and posterior rami (Figures 8, 9, 12, 13, 16, 17). The anterior ramus innervates the anterior and horizontal semicircular canals and utricle, and the posterior ramus innervates the posterior semicircular canal, saccule, and lagena (Popper and Fay 1977). As the rami course toward the medulla, they fuse to form a single ganglion and root, which enters the medulla together with the roots of the facial nerve. Upon entering the medulla (Figure 22D), the statoacoustic fibers occupy a ventral portion in the posterior lateral line lobe and form ascending and descending tracts.
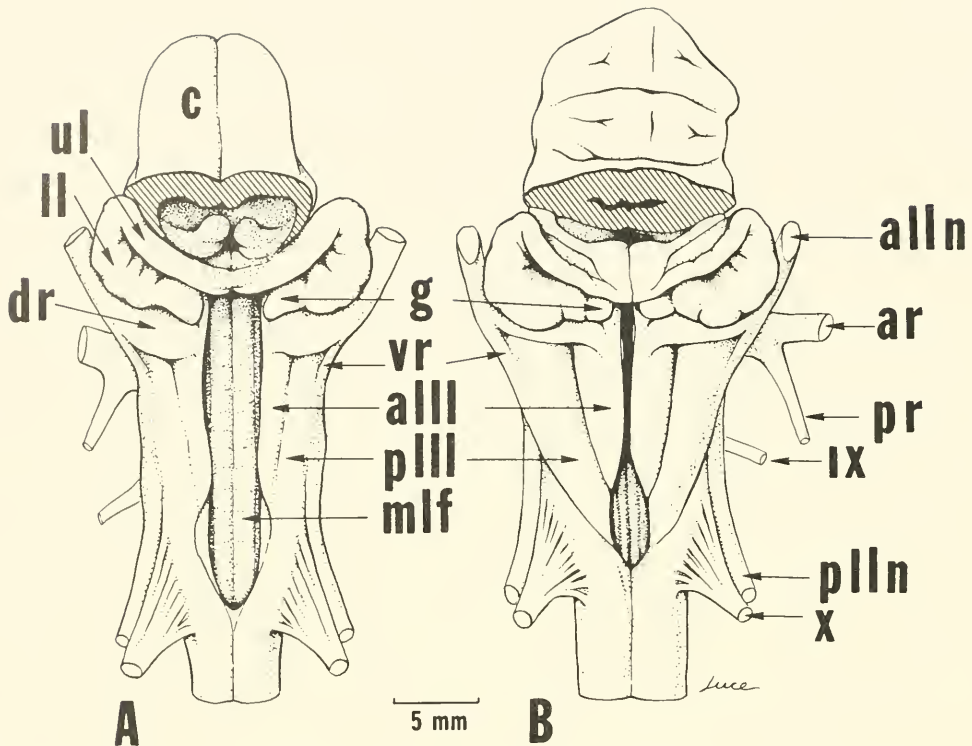
Figure 26   Dorsal view of the acousticolateralis area and brain stem in (A) *Squalus* and
(B) *Mustelus*; the caudal half of the cerebellar corpus is removed to show details of the
auricle. alll, anterior lateral-line lobe; alln, anterior lateral-line nerve; ar, anterior ramus of
statoacoustic nerve; c, corpus of cerebellum; dr, dorsal root of anterior lateral-line nerve;
g, granular cap of anterior lateral-line lobe; ll, lower leaf of auricle; mlf, medial longi-
tudinal fasciculus; plll, posterior lateral-line lobe; plln, posterior lateral-line nerve; pr,
posterior ramus of statoacoustic nerve; ul, upper leaf of auricle; vr, ventral root of
anterior lateral-line nerve; IX, glossopharyngeal nerve; X, vagal nerve.

The acousticolateralis area of the medulla can be divided grossly into
anterior and posterior lateral-line lobes (Figures 8, 9, 12, 13, 16, 17, 26).
Considerable variation occurs in the development of these lobes in cartila-
ginous fishes. Squalomorph (Figures 9, 26A) and squantinomorph sharks
are characterized by moderately developed lobes, whereas chimaeras and
galeomorph sharks show marked enlargement of both lobes with strongly
hypertrophied anterior lobes (Figures 8, 12, 13, 26B). Batoids do not exhibit
as much variation as sharks, but their level of lobe organization is most
similar to that of the galeomorph sharks (Figures 16, 17).

In all cartilaginous fishes the lateral and dorsal surfaces of the anterior and
posterior lobes consist of a neuropil layer, composed of unmyelinated axons
and a few stellate cells, which is termed the cerebellar crest (cc, Figures
21–23). Large Purkinje-like cells, deep to the cerebellar crest in each lobe,
send dendrites into the crest neuropil (Larsell 1967). In *Scyliorhinus*, the
dendrites of the Purkinje-like cells entering the crest are covered with spiny

processes. These dendrites may run directly toward the surface of the crest or pass obliquely, crossing much of the crest (Paul and Roberts 1977*a*). The unmyelinated fibers that make up most of the crest run rostrocaudally, passing among the dendrites of the more deeply located cells in a topographical relationship similar to the passing of parallel fibers through the Purkinje cells of the cerebellum.

Alnaes (1973) argued that the crest fibers are primary lateralis afferents, but Paul and Roberts (1977*b*, 1977*c*) have demonstrated that lateral-line input in *Scyliorhinus* does not directly activate the parallel fibers of the crest. Boord and Campbell (1977) have shown that the lateral-line nerves terminate in the nuclei deep to the crest, and in the auricle of the cerebellum, but not in the crest itself. In *Raja* the cerebellar crest of the anterior lateral-line lobe is crowned by a cap of granule cells termed the lateral granular layer (lg, Figures 16, 22). This layer consists mainly of small granule cells, among which are scattered a few much larger cells. The large cells are similar to the Golgi cells located in the cerebellar granular layer. The lateral granular layer not only caps the anterior lobe but also expands rostrally to cap the posterior lobe as well (Figures 22D, 23). A similar condition exists also in sharks (g, Figure 26), but here the granular layer is not visible laterally. It continues rostrally over the lateral line lobes as they run under the upper leaf of the auricle (ul, Figure 26). Following lesions of the lateral granular layer in *Raja*, degenerating axons are seen leaving the granular layer and coursing in the cerebellar crest, where they terminate on the dendrites of the deeper Purkinje-like cells of the lobe (R. L. Boord, personal communication.)

The bulk of the anterior and posterior lateral-line lobes consists of nuclei containing several cell types. Large bipolar and triangular cells on the ventral border of the crest send their superficial dendrites into the crest. Their deep dendrites extend across the neuropil of the deep nucleus. Within this nucleus, large, spherical neurons with widely branching dendrites and small, densely staining cells predominate. The core of the anterior lateral-line lobe receives electroreceptive afferents via the dorsal root of the anterior lateral-line nerve, and it is termed the dorsal nucleus of the anterior lateral line lobe (dn, Figures 21D, 22D).

Lesions of the anterior lateral line lobe in *Raja* (R. L. Boord, personal communication) reveal that its efferents first collect on the ventromedial edge of the lobe, then run ventrally and medially to where the bulk of the fibers cross the midline and enter the contralateral lateral lemniscus (ll, Figures 20, 23). Degenerating secondary lateralis fibers are traced rostrally in the lateral lemniscus; they terminate in the nucleus of the lateral lemniscus (nll, Figure 20A) and in the mesencephalic lateralis nucleus (mln, Figure 20D). To my knowledge, the mesencephalic lateralis nucleus has not been described in the literature before. Its projections and its homolog in sharks are unknown.

The organization of the posterior lateral-line lobe is more complex, as it receives projections from both lateralis nerves and is closely associated with the statoacoustic nuclei. The ventral root of the anterior lateral-line nerve

(vr, Figure 23A) occupies the dorsolateral surface of the posterior lateral-line lobe; both it and the posterior lateral-line nerve terminate among the more deeply located cells, termed nucleus intermedius (ni, Figures 21–23).

In *Raja*, a specialized plate of neurons is located within the nucleus intermedius and is termed nucleus x (Figures 22, 23). Nucleus x may be only an area of increased density within the intermedius, or it may be the source of efferent fibers to the statoacoustic and lateral-line nerve receptors. A similar but more ventrally located nucleus (nucleus B), has been described in *Squalus* and *Scyliorhinus* (Smeets and Nieuwenhuys 1976).

Without experimental data, it is at present impossible to define the ventral border of nucleus intermedius in *Raja* because both the ventral root of the anterior lateral-line nerve and the roots of the statoacoustic nerve enter at the same level (Figure 22D).

At this same level, an oval nucleus of large bipolar cells (mn, Figure 22D) is located medially among the entering fibers of the statoacoustic nerve. This nucleus is termed the magnocellular nucleus of VIII, and it is probably homologous to the lateral vestibular nucleus (Deiter's nucleus) in other vertebrates. A similar nucleus, termed the magnocellular vestibular nucleus, is known in *Squalus* and *Scyliorhinus* (Smeets and Nieuwenhuys 1976).

Upon entering the medulla, some fibers of the statoacoustic nerve terminate in the magnocellular nucleus, while others form ascending and descending tracts. The descending tract can be followed caudally into the obex region (de, Figures 21, 22). Small- to medium-sized triangular cells scattered among the descending statoacoustic fibers have been designated the descending nucleus of VIII. No experimental data exist on the projections of the statoacoustic nerve in *Raja*, but preliminary experiments on the stato-acoustic nerve in fetal *Squalus* (McCormick and Northcutt, unpublished observations) reveal a distinct descending pathway, which reaches a medullar level comparable to that shown in Figure 22A. A comparable statoacoustic pathway also exists in *Amia* (McCormick 1977) and can be traced to a medullar level comparable to that shown in Figure 21A in *Raja*.

Rostrally (Figure 23), as nucleus intermedius moves dorsally, a new nucleus (dn, Figure 21A, B, C) begins to develop. This nucleus I have tentatively identified as the dorsal nucleus of VIII in *Raja*, based on preliminary experiments in *Squalus*, and it may be homologous to part of the cochlear nuclear complex in land vertebrates. Smeets and Nieuwenhuys (1976) recognized a possibly similar cell group in *Squalus* and *Scyliorhinus* and termed it the superior vestibular nucleus.

Experimental study of the central connections of the lateralis and stato-acoustic systems in cartilaginous fishes is only beginning. However, lateralis input to the telencephalon has been demonstrated (Platt et al. 1974). Given the excellent sound detection and localization abilities of elasmobranchs (Popper and Fay 1977), it is likely that these fishes will possess well-developed ascending pathways related to these modalities. Perhaps one of the most interesting questions is whether elasmobranchs possess separate telencephalic projection areas for ampullary, ordinary lateral-line, and acoustic information. Such a possibility would have seemed impossible 10

years ago, but Knudsen (1977) has demonstrated such segregation at mid-brain levels in teleosts.

Cerebellum—The cerebellum of chondrichthians consists of a central unpaired corpus and laterally situated auricles (Figures 8, 9, 11–14, 16, 17, 26). Each auricle is divided into a dorsomedial upper leaf and a ventrolateral lower leaf continuous with the acousticolateralis region of the medulla (Figures 21–23, 26).

Earlier studies have emphasized that the size of the corpus is correlated with body size and is thus related to somatic musculature (Kappers et al. 1936, Aronson 1963), but my own observations do not support this contention. Animals of the same size (e.g., *Squalus acanthias*, *Mustelus canis*, and *Sphyrna tiburo*) exhibit the total range of cerebellar complexity seen among sharks. Shark cerebellar:body ratios (Table 3) also indicate that cerebellar size is not proportional to body size. *Mustelus* (6 kg) possesses a relatively larger cerebellum than *Carcharhinus* (36 kg).

The distribution of complex cerebellar foliation among elasmobranchs indicates that this condition has evolved a number of times independently. Chimaerids, squatinomorphs, and all squalomorph sharks examined possess a smooth corpus divided into anterior and posterior lobes (Figures 8, 9, 11A, 26A), suggesting that this condition is ancestral for cartilaginous fishes. A similar pattern is seen in heterodontids and scyliorhinids. However, lamniform and advanced carcharhiniform sharks possess a complexly convoluted corpus divided into three lobes (Figures 12-14, 26B). Batoids have evolved independently a complex corpus. The rajoids (Figure 16 and some torpediniforms possess a nonconvoluted corpus, while the rhinobatoid corpus is divided into three lobes (Figure 11B), as in many advanced sharks. The myliobatiforms (Figures 11C, 17) and some torpediniforms extend this trend and possess complex foliation, as do the carcharhinid sharks. However, different parts of the cerebellar cortex undergo hypertrophy in galeomorph sharks and myliobatiforms. The posterior cerebellar lobe in galeomorphs accounts for approximately half the cerebellar cortex (Figure 14C), while the anterior lobe accounts for half of the cortex in myliobatiforms (Figure 11C).

The functional significance of increased cerebellar volume in elasmobranchs is unknown, but it is very likely related to an increase in sensory inputs involved in motor control. Unfortunately there is little information on sensory pathways to the cerebellum. Ascending spino-cerebellar tracts to the corpus have been demonstrated (Hayle 1973b), but direct lateralis projections to the corpus, described by earlier studies (Kappers et al. 1936), have not been confirmed experimentally (Boord and Campbell 1977). Boord and Campbell demonstrated that the lateral-line nerves project directly to the auricle of the cerebellum, as do vestibular fibers.

Lesions of the cerebellar corpus do not result in locomotor impairment in *Squalus*, but lesioned animals do exhibit a decrease in general motor activity (Karamyan 1962). However, if lesions involve the cerebellar nucleus, the animals can not swim in a straight line and lack coordination of the various

body parts. Lesions of the auricle result in severe locomotor impairment in *Squalus* (Karamyan 1962). Animals with auricular lesions roll on the long axis and/or swim in circles, turning toward the side of the lesion. However, animals with bilateral auricular lesions swim normally.

Similar lesions in *Raja clavata* do not produce the same effects (Karamyan 1962). After removal of the corpus and cerebellar nucleus, no marked motor disorders are noted. Posture of the skates is normal, although some irregular contractions of fin segments are noted, and motor activity increases. Lesions involving the auricle produce marked postural changes, particularly in moving skates. These animals swim with rostrally elevated bodies, often swimming almost vertically.

Internal organization of the chondrichthian cerebellar cortex (Figure 20B) is similar to that of other vertebrates. Elasmobranchs possess all the cell types found in mammals, except for basket cells and a well-developed inhibitory plexus formed by Purkinje axon collaterals (Nicholson et al. 1969). Details of cerebellar neuronal anatomy and physiology have been recently summarized and reviewed by Nicholson et al. (1969), Paul (1969), and Tsukahara (1969).

Ebbesson and Campbell (1973) examined the cerebellar efferents in *Ginglymostoma* and demonstrated both ascending and descending cerebellar pathways like those found in other vertebrates. The Purkinje cells of the cerebellum of sharks, like those of other vertebrates, terminate on deep cerebellar nuclei, but direct projections to the vestibular nuclei or medulla, suggested by earlier studies (Kappers et al. 1936), were not confirmed. The cerebellar nuclei terminate in the lateral and medial medullar reticular formations, the red nucleus of the tegmentum, the trochlear and oculomotor nuclei, and the posterior dorsal thalamus. Thus, the cerebellum possesses descending projections, modulating the output of the reticular formation to the cranial nerve motor nuclei and to the spinal cord, and an ascending brachium conjunctivum that reaches dorsal thalamic levels. This last projection is particularly interesting, as it suggests that thalamo-telencephalic relay of cerebellar afference may exist. In other vertebrates the thalamo-telencephalic projection is to a motor area of isocortex. Demonstration of such a pathway would further strengthen the probability of telencephalic involvement in direct motor control suggested by Ebbesson's demonstration of direct telencephalic pathways to medullar nuclei (Ebbesson 1972).

Mesencephalon—The midbrain of chondrichthians consists of dorsal (optic tectum and torus semicircularis) and ventral (tegmentum) regions (Figures 2-5, 20, 27). The optic tectum consists of multiple laminae, frequently grouped into zones, but no agreement exists regarding the exact number (Table 5). I have used the nomenclature of Gerlach (1947) in this chapter and have grouped his layers into three zones: periventricular, central, and superficial (Figures 2-5, 20, 27).

The periventricular tectal zone (Figures 3, 4, 20, 27) consists of an un-myelinated fiber layer (layer 1) and two or more cellular laminae (layer 2). Pyriform cells predominate in this zone, and their apical dendrites branch
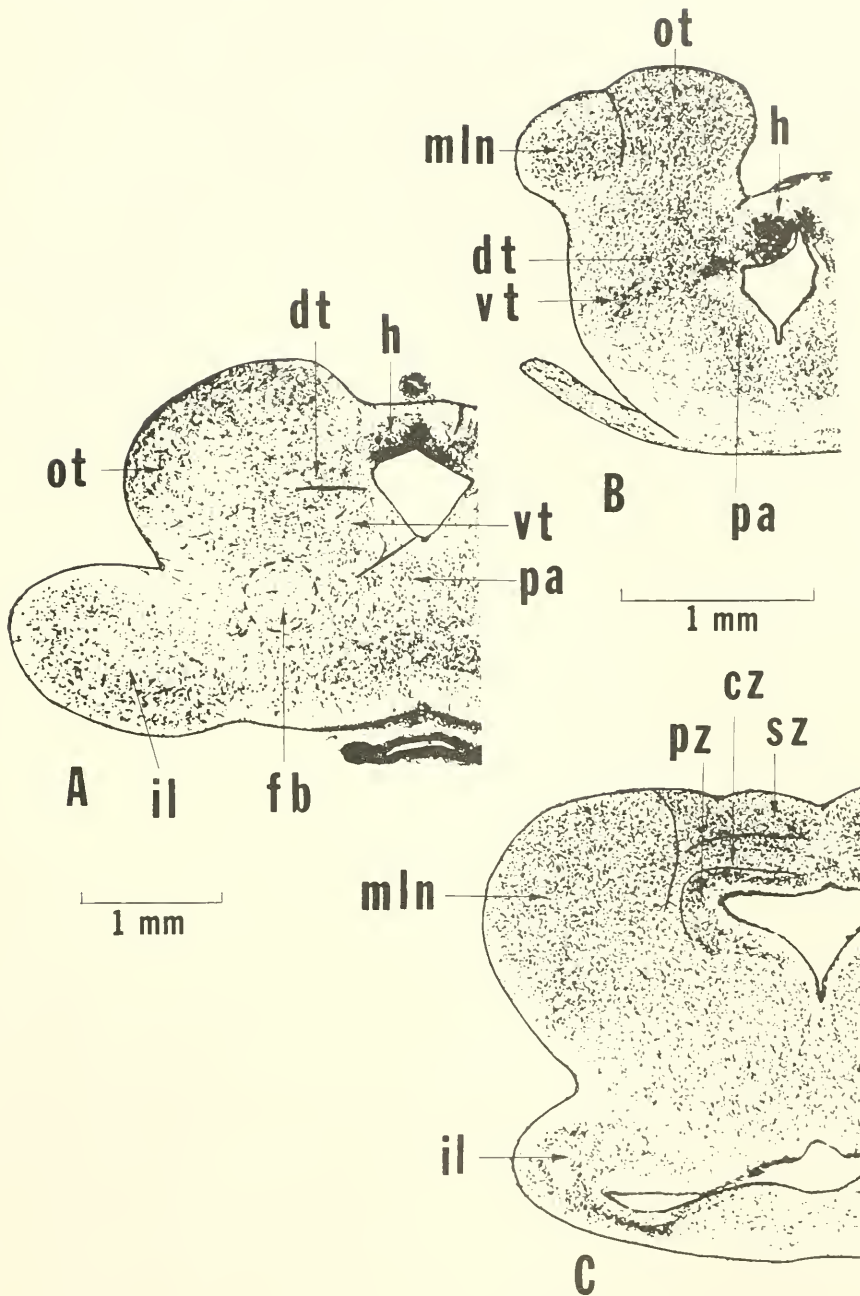
Figure 27 Transverser sections through the (A) right thalamus of *Raja eglanteria* and (B) thalamic and (C) mesencephalic levels of *Platyrhinoidis triseriata*. cz, central tectal zone; dt, dorsal thalamus; fb, forebrain bundles; h, habenular nuclei; il, inferior lobe of hypothalamus; mln, mesencephalic lateralis nucleus; ot, optic tectum; pa, preoptic area; pz, periventricular tectal zone; sz, superficial tectal zone; vt, ventral thalamus.

Table 5. Comparison of elasmobranch tectal nomenclatures.

Houser 1901	Kappers et al. 1936	Gerlach 1947	Leghissa 1962	Schroeder and Ebbesson 1975	Northcutt 1977
Central gray matter	Stratum cellulare internum	Layer 1 Periventrikuläre Schicht / Layer 2 Lamina cellularis interna	Strata 1–3 Zona grigia e fibrosa periventricolare	Layer E Stratum cellulare internum	Layers 1, 2 Periventricular tectal zone
Stratum medullare profundum	Stratum medullare internum	Layer 3 Lamina medullaris interna	Stratum 4 Zona biana e grigia media	Layer D Stratum medullare internum	Layers 3, 4 Central tectal zone
Deep zone	(Zona interna) / Stratum cellulare externum	Layer 4 Hauptzellenschicht		(Layer C, Zona interna) Stratum cellulare externum	
Middle zone	(Zona externa)	Layer 5 Opticusfasern		Layer B, Zona externa	Layers 5, 6 Superficial tectal zone
Superficial zone	Stratum medullare externum	Layer 6 kernarme Molekularschicht	Strata 5–8 Zona grigia e fibrosa esterna	Layer A Stratum medullare externum	

dorsally into the central and superficial zones (Leghissa 1962), contacting optic terminals of the superficial zone. The proximal dendritic shafts of the pyriform cells pass through the terminal zones of telencephalic, tectal commissural, and ascending spinal afferents, with which they probably form synapses. The axons of these cells arise from the soma, or a dendritic shaft, and enter the tectal efferent tract (layer 3) or the intertectal commissure, or else ramify within the central zone. All studies to date (Table 5) have recognized the same periventricular layers, though Houser (1901) excluded these layers (his central gray matter) from the tectum proper, as has traditionally been done in describing the mammalian optic tectum.

The central tectal zone consists of a deep fiber layer (layer 3) and a superficial layer of fibers and scattered neurons (layer 4). The neurons of layer 4 are termed large multipolar cells by Leghissa (1962) and send their dendrites into the superficial zone and their axons deep into layer 3.

Layer 3 is composed of axons that form the descending tectal efferents and intertectal commissural fibers (Ebbesson 1972). This layer also contains ascending fibers carrying sensory information into the tectum (Hayle 1973a). The tectum also projects rostrally to the pretectum and thalamus, terminating in nuclei that also receive retinal inputs and terminating caudally in the ipsilateral and contralateral medullar reticular formations (Ebbesson 1972). In other vertebrates with migrated tectal neurons, the tectal neurons that project rostrally are located in the superficial and central zones, while the cells that give rise to the descending tracts originate predominantly in the periventricular zone. Similar experiments have not been performed for cartilaginous fishes, but it is likely that they possess a similar organization.

It is evident from Table 5 that differences in interpreting the organization of the upper half of the tectum account for the variation in tectal nomenclature. It is difficult to establish the exact dorsal boundary of the central zone and the number of layers in the superficial zone. This difficulty stems from differences in interpreting the course of incoming optic fibers and from actual differences in development of layer 5 in sharks. Until recently, the optic fibers were believed to enter the tectum in layer 6, then turn ventrally to terminate in layers 4 and 5 (Kappers et al. 1936). However, experimental studies (Schroeder and Ebbesson 1975, Northcutt 1976) demonstrate that the optic fibers enter more deeply (layer 5 and upper part of layer 4) and turn dorsally to terminate predominantly, if not solely, in layers 5 and 6.

The pattern of optic terminals in the tectum appears different in squalomorph and galeomorph sharks (Figures 2–5). In squalomorphs, both optic fibers of passage and their terminals are located above the dense tectal cell layer; in galeomorphs, such as *Mustelus* (Figures 4, 5), *Ginglymostoma*, *Galeocerdo*, and *Negaprion* (Ebbesson and Ramsey 1968, Graeber and Ebbesson 1972a), the fibers of passage course in layers 4 and 5 and terminate in layers 5 and 6.

Present data are insufficient to determine whether the dense cell layer in *Squalus* is homologous to layer 4 or layer 5 in *Mustelus*. It is possible that both layers exist in *Squalus*, and that layer 5 is not as well developed as in

Mustelus and the other galeomorphs. However, if the upper half of the dense cellular band in *Squalus* is layer 5, then optic fibers terminate dorsal to the cell bodies in *Squalus*, whereas in *Mustelus* they terminate partly among the cell bodies of layer 5.

Traditionally, the ventral border of the superficial tectal zone is said to coincide with the ventral extent of the optic terminals (except in actino-pterygian fishes, where optic fibers enter the periventricular gray zone). I have retained this definition in recognizing the tectal zones in *Squalus* and *Mustelus*, though layer 5 is assigned to different zones on this basis. I believe this approach is preferable to redefining the boundaries of the zones; if zones are to be recognized at all, they should denote differences in tectal func-tions. However, this problem could be avoided by abandoning the concept of tectal zones and recognizing only individual tectal layers among chondrichthians.

Layer 5 consists of pyramidal and bipolar cells according to Leghissa (1962). Both cell types possess superficial dendrites that branch repeatedly in layer 6 and receive retinal input, as well as deeper dendrites that extend into layers 4 and/or 3. Many of the bipolar cells are likely to be multimodal, as they receive optic terminals superficially and other sensory input via their periventricularly directed dendrites.

Layer 6 consists of horizontal cells and scattered marginal cells (Leghissa 1962). The horizontal cells are bipolar neurons with horizontally radiating dendrites and an axon that terminates among the cells of layer 5 and pos-sibly layer 4. These cells are integrative in function, and their axons do not leave the tectum. The marginal cells are neurons with two to three dorsally directed dendrites that reach the tectal surface and axons that descend into the deeper tectal layers.

Leghissa (Table 5) has recognized more tectal layers than other workers (seven, excluding stratum 1, which denotes the ependymal layer). I have followed Gerlach's nomenclature here. However, a seventh layer can be recognized, as the optic terminals do not reach the surface (Figures 2–5), which is capped by a thin layer of unmyelinated fibers of unknown origin and could be termed a marginal layer.

Considerable variation exists among the tecta of chondrichthians, but too few genera have been examined to identify trends, particularly for the batoids. The tectum of the chimaerid *Hydrolagus* is distinctly different from that of elasmobranchs. Eighty to ninety percent of the tectal neurons are located periventricularly, and the neurons are in contact with the ventricular ependyma so that a deep fiber layer (tectal layer 1) does not exist as it does in elasmobranchs. The most ventral periventricular layer consists of neurons, seven to eight cells thick, capped by three additional periventricular layers (one to two cells thick) separated by thin fiber layers. The central tectal zone in chimaeras consists of heavily myelinated fibers (homologous to the elasmobranch layer 3) arranged in vertical columns with a few cells scattered between the columns. The superficial tectal zone accounts for approximately half the thickness of the tectum and consists of four distinct layers: margi-nal, superficial optic fibers, neuropil and deep optic fibers.

The visual system is well developed in *Hydrolagus*, and the entering optic fibers divide the superficial tectal zone into distinct layers. A thin series of optic fascicles runs horizontally, just beneath the tectal surface; these fascicles are covered by unmyelinated nonretinal fibers, as in elasmobranchs. However, most of the optic fibers enter more deeply. Thus, a distinct neuropil of dendritic processes, unmyelinated fibers, and some cells exists between the small superficial optic fiber component and the massive deep optic fibers. This neuropil is probably the major site of optic terminals, but experimental studies are needed to confirm this speculation.

The tectum of *Notorynchus* is almost identical to that of *Squalus* (Figures 2, 3). Both possess deep periventricular laminae (one to two cells thick) and a centrally located cellular plate that is possibly homologous to tectal layers 4 and 5 of *Mustelus*. Clearly most of the tectal neurons are restricted to the central tectal zone, thus differing from *Hydrolagus* as well as the galeomorph sharks (Figures 4, 5). All galeomorph tecta examined to date, except possibly *Scyliorhinus*, are similar to *Mustelus*. The tectum of *Scyliorhinus* appears intermediate between that of squalomorphs and galeomorphs, but experimental study of the retinal projections is needed to confirm this observation.

The tecta of *Platyrhinoidis* and *Raja* (Figures 20, 27), like those of galeomorph sharks, have the highest cell concentrations in the superficial zone, and can be distinguished from the tecta of all sharks by a periventricular zone without distinct cellular laminae, and by a more restricted mediolateral axis. However, the same tectal layers can be recognized in batoids as in galeomorph sharks; and the optic fibers predominantly enter the deeper half of the superficial zone and terminate more dorsally (Northcutt and Boord, unpublished observations).

The range of tectal variation observed in chondrichthians suggests that chimaeras possess the most primitive tectal pattern, and that the tecta of elasmobranchs are characterized by extensive cellular migrations away from a thick periventricular cellular plate. Among elasmobranchs, squalomorphs probably exhibit the most primitive tectal pattern, characterized by a dense cellular plate in or on the border of the central tectal zone. Both galeomorph sharks and batoids possess tecta with hypertrophied superficial tectal zones of high cell density. Additional studies will likely reveal that the advanced tectal condition has evolved independently in galeomorphs and batoids.

As the periventricular tectal zone is traced laterally in chimaeras and sharks, the cellular laminae lose their compactness and form a scattered nucleus called the torus semicircularis (Figures 3B, 5). I have been unable to recognize a cytologically distinct torus in batoids, and experimental studies are needed to determine the homologous population. The connections of the torus are unknown in chondrichthians, but in other anamniotes a similarly situated nucleus receives auditory and lateralis afferents (Page 1970, Knudsen 1977), and toral efferents have been traced to thalamic and reticular populations.

The midbrain floor, or tegmentum, passes over from the hindbrain through the isthmus, a transitional region that is sometimes recognized as a separate brain division. The isthmus, or caudal tegmentum (Figure 20A), is

distinguished by the most rostral continuation of the cerebellar granular layer dorsomedially, and by nucleus isthmi dorsolaterally (ni, Figure 20A). Nucleus isthmi consists of small spherical and bipolar cells oriented horizontally relative to the ventricular sulcus. The connections of nucleus isthmi in chondrichthians are presently unknown. In other vertebrate species, nucleus isthmi receives a topographically organized projection from the optic tectum and projects it back onto the cells of the central tectal zone. The function of this tectal feedback system is related to vision, but its exact significance is unknown.

At these same caudal tegmental levels, a ventral midline nucleus, the nucleus interpeduncularis (in, Figures 3B, 5B, 20) is recognized. This nucleus receives afferents from the habenular nuclei via the fasciculus retroflexus (fr, Figures 2C, 5A). The efferents of the interpeduncular nucleus are undetermined in cartilaginous fishes, but this nucleus forms extensive descending medullar projections in other vertebrates.

More rostrally, the medial tegmentum is characterized by a nucleus of large scattered neurons, nucleus interstitialis (in, Figures 3A, 5A). The afferents to this nucleus are unknown, but the axons of its cells form the most rostral component of the medial longitudinal fasciculus, and connections with spinal neurons are known in *Dasyatis* (R. B. Leonard, personal communication).

In sharks, the dorsocentral tegmental region is occupied by the intercollicular nucleus (ic, Figures 3, 5). In batoids, a distinct intercollicular nucleus was not recognized, but may be represented by the ventral portion of the caudal central tectal zone (cz, Figure 20B, C). The intercollicular nucleus receives spinal input in sharks (Ebbesson 1972). Its efferents are undetermined in chondrichthians, but in other vertebrates it projects rostrally into the thalamus, forming an ascending somatosensory pathway.

The ventrocentral tegmentum consists of two nuclei: a ventrolateral cell group, the lateral tegmental nucleus; and a dorsomedial cell group, nucleus ruber. The lateral tegmental nucleus (lt, Figure 20B) consists of small spherical cells in *Raja,* and spinal input to this nucleus occurs in *Scyliorhinus* (Hayle 1973a). Its efferents are unknown, but its topographical position suggests that it may be homologous to the substantia nigra of other vertebrates. If so, it will likely receive tectal input and possess reciprocal connections with the dorsal striatum of the telencephalon.

Nucleus ruber (t, teg, Figures 3B, 20C) consists of medium-sized fusiform and triangular cells. It receives ascending input from the cerebellar nucleus in *Ginglymostoma* (Ebbesson and Campbell 1973) and gives rise to a crossed descending pathway that reaches spinal levels (R. B. Leonard, personal communication). In other vertebrates, nucleus ruber also receives afferents from the telencephalon; a similar projection may exist in elasmobranchs; Ebbesson (1972) has charted a heavy terminal field in the region of nucleus ruber in *Ginglymostoma*, following telencephalic lesions. In mammals, telencephalic projections to nucleus ruber arise in primary motor cortex, and the cortico-rubrospinal pathway activates contralateral flexor motor neurons and inhibits contralateral extensor neurons. Thus, the elasmobranch

telencephalon may modulate motor movements, in part, via a similar cortico-rubrospinal pathway.

In batoids, the rostrolateral tegmentum is dominated by a large spherical nucleus that receives lateralis input via the lateral lemniscus; it is termed the mesencephalic lateralis nucleus (mln, Figures 20D, 27). This nucleus consists of medium-sized bipolar and triangular cells scattered among ascending fascicles of the lateral lemniscus. The cell density is greatest along the lateral half of the nucleus. Its efferents are unknown, but they likely project to the telencephalon via thalamic relay. A comparable nucleus has not been identified in sharks, but the midbrain cell group, nucleus profundus mesencephali (np, Figures 2D, 3A, 5A), in *Squalus* and *Mustelus* may be homologous to the mesencephalic lateralis nucleus of batoids.

More ventrally, a basal optic nucleus occupies the ventrolateral tegmental floor (bon, Figures 3B, 5B, 20C). This nucleus receives optic fibers from the contralateral eye, and its efferents are unknown in elasmobranchs. In other vertebrates, the basal optic nucleus projects to the vestibulo-cerebellum, mediating optokinetic nystagmus (Lazar 1973, Brauth and Karten 1977, Karten et al. 1977).

Diencephalon—The diencephalon of chondrichthians consists of three divisions: epithalamus, thalamus, and hypothalamus. The epithalamus is formed by the habenular nuclei, the habenular commissure, and a complex series of afferent and efferent pathways (stria medullaris complex) related to the habenular nuclei. Little variation is discernible among the various species. The habenular nuclei consist of dorsal and ventral divisions (Figures 2, 4, 27). The ventral habenular nucleus fuses across the midline below a much shortened habenular commissure. The main efferent pathway of the habenular nuclei (fasciculus retroflexus) terminates caudally in the interpeduncular nucleus (Figures 3, 5, 20). No experimental studies exist on the afferent pathways to the habenula of cartilaginous fishes.

The epithalamus of many vertebrates is characterized by two dorsal evaginations, the epiphysis (pineal organ) and paraphysis (parietal organ), in addition to the habenular nuclei and related tracts. The chondrichthian epiphysis is well developed and runs rostrally from the habenular area to end beneath the roof of the skull over the telencephalon. The epiphysis of sharks consists of both ganglion and sensory cells (Studnička 1905), and electron microscopic studies have shown that the sensory cells possess outer segments similar to retinal cone photoreceptors (Rüdeberg 1969). Hamasaki and Streck (1971) demonstrated that the epiphysis of *Scyliorhinus* is photosensitive to very low levels of illumination (theoretically, levels as low as those associated with a full moon) and that such illumination elicits both slow potentials and inhibition of spike activity. Similar results are known for a wide variety of vertebrates, and the epiphysis is believed to function in control of skin color and circadian rhythms.

The thalamus of chondrichthians is divided into dorsal (dt) and ventral (vt) divisions (Figures 2–5, 27). The thalamus of chimaeras and squalomorph sharks

is similar to that of many other anamniotic vertebrates in consisting of prominent periventricular cell groups and a more lateral sparsely celled neuropil (Figure 2). In contrast, the thalamus of galeomorph sharks and batoids (Figures 4, 5, 27) is characterized by marked thickening of the thalamic wall and cellular migration away from the ventricle. The galeomorph thalamus not only is larger than that of squalomorphs, but is also better differentiated into distinct nuclei similar to the thalamus in birds and reptiles.

Experimental studies on retinal projections in sharks (Ebbesson and Ramsey 1968, Graeber and Ebbesson 1972*a*, Northcutt 1976) and skates (Northcutt and Boord, unpublished observations) reveal that the optic projections are entirely crossed and that both dorsal and ventral thalamic nuclei receive optic terminals (Figures 2–5). The decussating optic fibers form a lateral or marginal optic tract that courses dorsally, terminating in the lateral neuropil of the dorsal and ventral thalamus (Figures 2A, 4A).

The rostral dorsal thalamus of *Squalus* consists of two major laminae: a thin medial lamina and a larger densely packed lateral lamina with a sparsely celled neuropil that receives retinal input (Figure 2A). Similar divisions can be recognized in *Mustelus*, but there the thalamic wall is much thicker and the retino-recipient zone contains as many neurons as the more medial thalamic lamina (Figure 4A).

More caudally, the lateral lamina of the dorsal thalamus is replaced by a new neural population embedded in the optic tract and termed the superficial pretectal nucleus (sp, Figures 2, 4, 20D, 27). Unlike the lateral lamina, the rostral medial lamina of the dorsal thalamus continues caudally and expands ventrally, assuming the shape of an inverted V (Figures 2B, 4B). Further caudally, this lamina is finally replaced by nucleus interstitialis at midbrain levels (Figure 5A). The medial lamina forms the bulk of the dorsal thalamus; it is not a homogeneous plate, but can be divided into four or five distinct nuclei based on cell size and packing densities. However, determination of the exact number and the boundaries of these nuclei requires further experimental information.

Only two distinct dorsal thalamic nuclei (rostral thalamic and superficial pretectal nuclei) receive visual input, but the ventral thalamus receives retinal fibers throughout its entire lateral, rostrocaudal extent (Figures 2–5). Rostrally, the ventral thalamus also consists of two cell groups: a dorsal circular nucleus and a ventral, more oblong nucleus with more densely packed cells (Figure 2A, B). The ventrolateral edge of the ventral nucleus juts laterally around the edge of the forebrain bundles (Figures 2A, 4A) and may represent an entopeduncular nucleus as in many other nonmammalian vertebrates. The ventral thalamus extends far caudally beneath the optic tectum (Figures 2D, 4B), and as its caudal border is approached (Figure 20D) retinal fibers terminate medially almost in contact with the periventricular cell bodies. More caudally, the ventral thalamus is replaced by the midbrain tegmentum, which is first recognized as a medial and dorsal continuation of the hypothalamus (Figure 3A).

The caudal dorsal thalamus or pretectum (Figures 2C, 4B, 20D) consists of three cellular groups lying dorsal and lateral to the subcommissural organ and

posterior commissure. These pretectal nuclei correspond topologically to the rostral continuation of the superficial, central, and deep, or periventricular, tectal zones; therefore, they have been named according to these zones.

At rostral thalamic levels (Figure 2B), a second optic pathway, the medial optic tract, forms by splitting from the marginal optic tract and courses dorsomedially, where it divides into dorsal and ventral fascicles. The dorsal fascicle courses over the intertectal commissure and enters the rostral tectum (Figure 2B). The ventral fascicle continues caudally through the pretectal area, terminating among the cells of both the central and periventricular pretectal nuclei (Figures 2C, D, 4B). Cells of both the periventricular pretectal nucleus and tectum may receive retinal input via the ventral fascicle of the medial optic tract, as both cellular areas possess apical dendrites entering the central pretectal zone.

All of the thalamic and pretectal nuclei receiving retinal input also receive ascending tectal input (Ebbesson et al. 1972). Most of the remaining dorsal and ventral thalamic nuclei, medial to the retinal and tectal recipient zones, receive ascending cerebellar and spinal inputs (Ebbesson et al. 1972, Ebbesson and Campbell 1973). Thus the thalamus of sharks receives a wide range of ascending sensory pathways, and considerable separation of sensory modalities appears to exist.

The thalamus of sharks is also known to give rise to sizable ascending pathways that terminate primarily in the central nucleus of the telencephalon (Ebbesson 1972, Schroeder and Ebbesson 1974). The exact origins of these ascending thalamo-telencephalic pathways are unknown; but it is established that thalamic projections are to the contralateral telencephalic hemisphere, rather than to the ipsilateral hemisphere as in other vertebrates.

At present, nothing is known about the pretectal efferents in chondrichthians. However, in other vertebrates the pretectum forms complex connections with several different brain regions, such as the tectum, corpus of the cerebellum, tegmentum, and the more rostral dorsal thalamic nuclei, and has been implicated in such diverse functions as eye—head coordination and visual detection of potential predators.

Little is known about the third division of the diencephalon, the hypothalamus. It consists of a rostral preoptic area, a central or tuberal area including the inferior lobes, and a caudal or posterior hypothalamic area (Figures 2-5, 20, 27). Retinal projections are known to the rostroventral preoptic area (Graeber and Ebbesson 1972a, Northcutt 1976); See Figure 2B, C. Telencephalic input to the preoptic area and inferior lobes has also been demonstrated (Ebbesson 1972). The lateral lobes form the bulk of the chondrichthian hypothalamus, and considerable variation exists in their organization.

In chimaeras, sharks, and some batoids (*Platyrhinoidis*) the inferior lobes are characterized by extensive lateral recesses of the third ventricle, and the lobar nuclei are organized mainly as periventricular laminae (Figures 2, 3, 5, 27C). In the advanced batoids, the lateral recesses are reduced, and distinct nuclear groups replace the periventricular laminae (Figures 20D, 27A). The inferior lobes have been implicated in feeding behaviors (Demski 1977) and in the

control of the melanophore-stimulating hormone (Wilson and Dodd 1973). Electrical stimulation of the lobes in unanesthetized, free-swimming nurse sharks (*Ginglymostoma*) evoke biting and mouthing of food (Demski 1977), and Wilson and Dodd (1973) have demonstrated that the inferior lobes of *Scyliorhinus* are characterized by extensive aminergic innervation and exert inhibitory control of the release of the melanophore-stimulating hormone.

Nothing is known about the efferent hypothalamic pathways in chondrichthians, but telencephalic, medullar, and perhaps spinal pathways exist, as in other vertebrates.

Telencephalon—The chondrichthian telencephalon, like that of other vertebrates except actinopterygian fishes, consists of paired evaginated cerebral hemispheres and a caudal telencephalon medium (Figures 8, 9, 12, 13, 16, 17). The telencephalon medium in all vertebrates is a part of the embryonic forebrain that is not carried laterally into the evaginating or everting hemispheres. In all vertebrates, the olfactory bulbs arise by a secondary evagination from the cerebral hemispheres. Such is the case in elasmobranchs; however, the olfactory bulbs and their peduncles (olfactory tracts) arise far laterally (Figures 7C, D, 9, 12, 13, 16, 17).

The olfactory bulbs in chimaeras (Figure 8) arise rostrally from the frontal pole of the hemispheres and have distinct dorsal and medial divisions, which appear to receive their input from dorsal and ventral halves, respectively, of the olfactory organ. The olfactory bulbs of most sharks and skates (Figures 9, 12, 13, 16, 17) possess distinct medial and lateral divisions. Norris and Hughes (1920) reported that the medial division of the olfactory bulb in *Squalus* receives input from the medial and lateral lamellae of the olfactory organ, while the lateral division receives input from only the lateral lamellae. Daniel (1934), however, argued that the medial and lateral divisions of the bulb receive input from the medial and lateral lamellae, respectively. Experimental studies are needed to determine the olfactory epithelium's exact pattern of projection onto the bulb. However, differences in bulbar divisions and their peduncular development among elasmobranchs suggest important topographical organization.

Nieuwenhuys (1967) reviewed earlier literature on the histological organization of olfactory bulbs in chondrichthians and recognized four distinct bulbar layers: an outer layer of primary olfactory fibers, a glomerular layer formed by the dendrites of deeper mitral and large triangular cells, a mitral layer formed by the mitral cell bodies and the secondary olfactory fibers, and a deep granular cell layer (Figure 7C, D). The granular cells of chondrichthians, like those of lampreys, possess axons, and the secondary olfactory fibers arise not only from mitral cells, as in most vertebrates, but also from large triangular cells within the granular layer.

Until recently, all parts of the elasmobranch telencephalon were believed to receive secondary olfactory fibers from the olfactory bulb (Bäckström 1924, Kappers et al. 1936, Aronson 1963). However, new experimental studies on sharks and skates reveal that olfactory projections to the telencephalon of elasmobranchs are as restricted as those in land vertebrates

(Ebbesson and Heimer 1970, Bruckmoser and Dieringer 1973, Ebbesson and Northcutt 1976).

In sharks, two main telencephalic areas receive ipsilateral secondary olfactory fibers: the lateral pallium (lateral olfactory area of Ebbesson and Heimer, 1970), and a lateral portion of area superficialis basalis (Figures 6, 10). In *Raja eglanteria*, the secondary olfactory projections (Northcutt and Boord, unpublished observations) are confined to the lateral hemispheric wall, terminating in the lateral pallium (lp, Figure 15B, C, D) and in a more medial nucleus termed nucleus a (a, Figure 15B).

Secondary olfactory fibers do not extend to large portions of the telencephalon in sharks and skates. However, it could be argued that the telencephalon is concerned mainly with olfactory information if the centers receiving direct olfactory input projected widely within the telencephalon. Ebbesson (1972), though, has shown that the lateral olfactory area in *Ginglymostoma* reveals only limited telencephalic projections. It does project to the ipsilateral area superficialis basalis and, quite sparsely, to the contralateral lateral olfactory area and area superficialis basalis, thus avoiding the bulk of the telencephalon.

At present, it is difficult to compare the overall pattern of elasmobranch olfactory organization to that of other vertebrates. Sharks and skates are similar to mammals in that the secondary olfactory projections are ipsilateral and terminate in only a portion of the ipsilateral hemispheric wall; olfactory projections are bilateral in all other anamniotes. This difference is particularly striking when elasmobranchs are compared to actinopterygian fishes, which reveal bilateral olfactory projections to the telencephalon, as well as direct projections to the hypothalamus (Finger 1975, Northcutt and Braford 1977).

However, the main olfactory target of elasmobranchs, unlike that of mammals, does not project outside the telencephalon but to other telencephalic areas, as in amphibians and reptiles. Thus, the overall pattern of olfactory organization in elasmobranchs is unique among vertebrates; yet certain features of that pattern characterize many other vertebrate groups.

There are no experimental data for olfactory projections in chimaerids. In fact, earlier descriptive studies do not agree about the extent of the secondary olfactory fibers or their probable targets. Holmgren (1922) and Kuhlenbeck and Niimi (1969) established the pallio-subpallial boundary high on the lateral telencephalic wall (position 1 in Figure 28); Faucette (1969a, 1969b) established a boundary much lower on the telencephalic wall (position 2 in Figure 28). Thus, two different cell groups in the chimaerid telencephalon have been interpreted as the homolog of lateral (piriform) pallium. Holmgren (1922) and Kuhlenbeck and Niimi (1969) argued that the cell group immediately dorsal to position 1 was the lateral pallium. Faucette (1969a, 1969b) argued that a more ventral cell group (cell group w in Figure 28) was the lateral pallium. In *Hydrolagus*, a compact, heavily myelinated bundle can be traced from the lateral surface of the olfactory bulb, and must represent the main, if not sole, secondary olfactory pathway (ot, Figure 28). My own analysis of the telencephalon of *Hydrolagus* suggests, as did
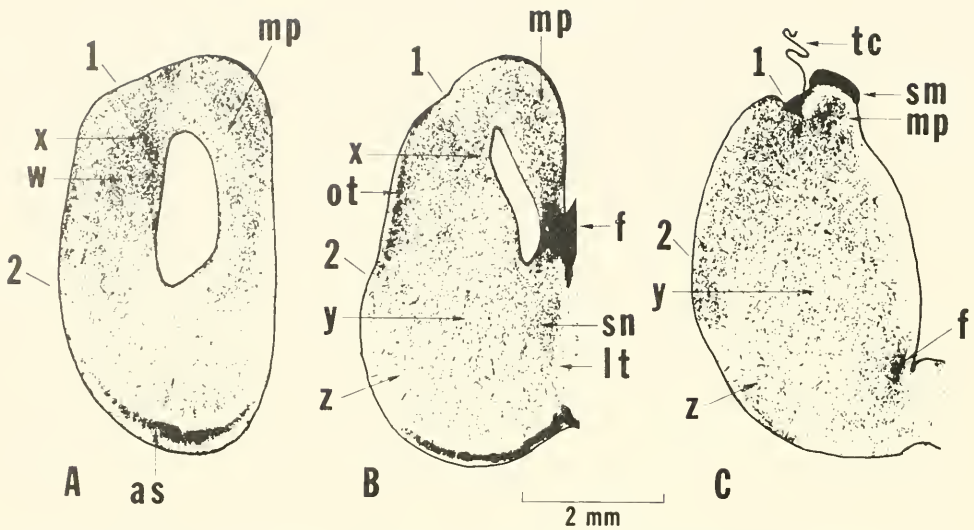
Figure 28   Transverse sections through (A) rostral, (B) mid, and (C) caudal levels of the right telencephalic hemisphere of the ratfish, *Hydrolagus colliei*. The levels of these sections are indicated in Figure 8. as, area superficialis basalis; f, fornix; lt, lamina terminalis; mp, medial pallium; ot, olfactory tract; sm, stria medullaris; sn, septal nuclei; tc, tela chorioidea; w, cell group w (possible lateral pallium); x, cell group x (possible dorsal pallium); y, cell group y (possible striatum); z, cell group z (possible pallial amygdala); 1, pallial-subpallial boundary of Holmgren (1922) and Kuhlenbeck and Niimi (1969); 2, pallial-subpallial boundary of Faucette (1969a, 1969b).

Faucette, that the pallio-subpallial boundary is low on the lateral telencephalic wall. However, it is quite possible that the cell group Faucette (1969a, 1969b) recognized as lateral pallium (cell group w, Figure 28A) is actually part of the dorsal pallium or a nonolfactory segment of the lateral pallium. Cell group w in *Hydrolagus* is characterized by high cell density, and a well-developed pathway of myelinated fibers courses to and/or from the cell group along the ventrolateral telencephalic wall, in a pattern reminiscent of the lateral forebrain bundle in other vertebrates. Thus, cell group w may have connections with the dorsal thalamus and may represent part of a pallial development similar to the dorsal ventricular ridge of land vertebrates. I believe that the more sparsely celled area, which lies lateral to cell group w and receives secondary olfactory fibers, is a more likely homolog of the lateral pallium.

Holmgren (1922) believed that a more dorsal cell group represented the lateral pallium in chimaeras, but he suggested that the main olfactory target was a more ventral area (cell groups w and x, Figure 28), which he termed the lateral olfactory nucleus. Kuhlenbeck and Niimi (1969), however, argued that this telencephalic region in chimaeras is homologous to the striatum of other vertebrates.

Finally, all studies to date have interpreted a ventrally located cell plate (as, Figure 28) in the telencephalon of chimaeras as homologous to the olfactory tubercle of other vertebrates and have assumed that the heavily

myelinated tract that courses among the cells of this ventral plate is second-
ary olfactory fibers. Earlier elasmobranch studies also assumed that this
ventral plate received olfactory projections, but recent experimental studies
have not confirmed these assumptions. Similar experimental studies do not
exist for chimaeras, but I have not been able to trace the myelinated fibers
of the ventral plate back into the olfactory bulb, and I suspect that these
fibers, like those of elasmobranchs, are not secondary olfactory fibers.

The details of these projections are still preliminary, but they suggest that
Finally, analyses of the number of distinct telencephalic areas in chon-
drichthians and their homologies with other vertebrates have been handi-
capped by the extensive cell migrations, the loss of distinct cell-free zones,
and the reduction in ventricular volume that characterize galeomorph sharks
and batoids (Figures 6, 7, 15). Holmgren (1922) stressed embryological
studies as a fruitful approach to these problems, and fortunately he chose to
study one of the simplest shark brains (*Squalus acanthias*). I have examined
embryos of *Squalus* as well as the telencephalic histology of a number of
adult sharks and batoids. Based on these studies is my belief that chon-
drichthians, like many other vertebrates, possess three main roof (pallial)
formations (Figures 6, 7, 10, 15, 28). The lateral pallium (lp) receives the
main olfactory input and is probably homologous to the lateral pallium of
land vertebrates. The dorsal pallium (dp and cn) is divided into inner and
outer laminae (Figures 6, 7, 10, 15). In elasmobranchs the outer lamina,
unlike that of other vertebrates, continues across the midline, forming an
interhemispheric bridge (Figures 6, 7, 10, 15). The inner lamina undergoes
extensive evolution in elasmobranchs. In *Notorynchus*, the inner lamina of
the dorsal pallium is poorly developed, forming only a slight bulge in the
roof of the lateral ventricle (Figure 10C, D). In squalids, the inner lamina is
better developed and caudally forms a thickened mass termed the central
nucleus by Ebbesson (1972) (Figure 6E). In squalids the central nucleus does
not fuse at the midline but remains a distinct and separate cell group. In
galeomorphs, the central nucleus is extensively hypertrophied, resulting in a
massively thickened interhemispheric bridge (Figure 6D, F). The central nu-
cleus receives substantial ascending sensory projections from the thalamus of
the diencephalon (Ebbesson and Schroeder 1971, Ebbesson 1972, Schroeder
and Ebbesson 1974). This pallial center is now known to receive visual,
lateralis, and trigeminal sensory inputs (Cohen et al. 1973, Platt et al. 1974).

The details of these projections are still preliminary, but they suggest that
the central nucleus is divided into a number of functional areas, rather than
consisting of a single cellular population with multimodal properties. A
number of distinct cytological subdivisions can be recognized in that pallial
region termed the central nucleus (Figures 6, 7, 10, 12, 15, 17), and further
studies will likely demonstrate that differences in the development of this
pallial complex are correlated with differential sensory specialization among
elasmobranchs.

The dorsal pallium of batoids is more like that of galeomorph than
squalomorph sharks. All batoids examined to date have reduced lateral ven-
tricles and well-developed central nuclear complexes. *Platyrhinoidis* (Figure 7)
reveals the simplest pallial development among batoids and is most

comparable in this respect to intermediate galeomorphs. The pallium of *Raja* (Figure 15) reflects only an intermediate level of the complexity reached by batoids. Comparison of a series of sharks and a series of batoids, *Notorynchus—Squalus—Mustelus—Sphyrna* and *Platyrhinoidis—Raja—Dasyatis*, suggests that parallel development of the dorsal pallium characterizes the advanced sharks and batoids. More detailed studies are needed to determine if comparable dorsal pallial regions receive the same sensory inputs and have hypertrophied in both sharks and batoids.

It is now impossible to determine whether chimaeras have similar pallial specializations. Analyses by Holmgren (1922) and Kuhlenbeck and Niimi (1969) suggest that the chimaerid pallium is restricted in volume and possesses neither interhemispheric neural bridges nor pallial specilizations comparable to those of elasmobranchs. On the other hand, Faucette (1969b) interpreted a dorsolateral cell group (cell group x, Figure 28) as homologous to part of the dorsal ventricular ridge—an avian and reptilian pallial complex that receives thalamic sensory projections from a wide array of different sensory systems. The dorsolateral cell groups (groups x and/or w, Figure 28) of the chimaerid telencephalon may represent specializations of the dorsal and/or lateral pallia, an interpretation consistent with their topography. However, all interpretations are merely speculative until experimental information establishes the connections and functions of the various telencephalic cell groups in chimaeras.

A more medial pallial group (mp) borders the dorsal pallium in chondrichthians (Figures 6, 7, 10, 15, 28), and in elasmobranchs it fuses across the interhemispheric bridge (Figures 6, 7, 10, 15). Nothing is known about its connections, but its topography suggests strongly that it is homologous to the medial pallium (hippocampal complex) of land vertebrates.

Discussions of other vertebrate pallia frequently include an additional telencephalic area—the pallial or cortical division of the amygdala. The vertebrate amygdaloid complex is not a single nucleus, but consists of two to eight major nuclei divided into basal (subpallial) and pallial divisions. The topographical positions of these nuclei are easily recognized in early embryonic stages of mammals, or in vertebrates with simple pallial development, such as amphibians (Northcutt 1974). The pallial division of the amygdala arises laterally as a caudoventral continuation of the lateral pallium. It expands medially, occupying the caudal floor of the telencephalon, and fuses with the rostral preoptic area of the hypothalamus. As the pallial amygdala courses caudally and medially, it lies adjacent to a second cell group, the basal amygdala which arises medially beneath the septal nuclei and also courses caudally. Upon reaching the lamina terminalis, however, the basal amygdala fuses with the contralateral basal amygdala. Thus, the simplest pattern of amygdaloid development occurs in amphibians (Northcutt 1974) and consists of a mediobasal amygdaloid cell group in the shape of a C (whose arms are directed rostrally and whose base runs in the lamina terminalis) and a more lateral pallio-amygdaloid cell group running caudally from the lateral pallium through the lamina terminalis to the preoptic area of the hypothalamus.

A similar pattern of amygdaloid development may exist in chondrichthians. A more scattered cell group can be recognized as a ventral continuation of the lateral pallium in chimaeras (cell group z, Figure 28) and in elasmobranchs (cell group a, Figures 10, 15). In both sharks and skates, nucleus a receives secondary olfactory fibers and can be traced caudally to where it eventually replaces a more medioventral cell group, area superficialis basalis.

In land vertebrates, the pallial division of the amygdala receives secondary olfactory fibers from a specialized part of the olfactory system (the vomeronasal organ), whereas the basal amygdala receives substantial input from the lateral pallium. A similar condition exists in chondrichthians. The area superficialis basalis lies adjacent to nucleus z of chimaeras (nucleus a of elasmobranchs) and can be traced caudally into the lamina terminalis where it clearly fuses across the midline in chimaeras (Figure 28B) and in *Notorynchus*. Area superficialis basalis does not receive a direct olfactory input. However, it does receive massive input from the olfactory-dominated lateral pallium (Ebbesson 1972). These relationships suggest that nucleus a (nucleus z in chimaeras) is homologous to the pallial amygdala of land vertebrates and that area superficialis basalis is homologous to the basal amygdala of land vertebrates. In land vertebrates these amygdaloid divisions have different histochemical properties, as well as differential projections to widely scattered forebrain centers, including the hypothalamus. Thus the proposed homologies with elasmobranchs can be tested by histochemical studies. If these studies confirm the proposed homologies, experimental tracing methods should reveal well-developed hypothalamic connections with nucleus a and area superficialis basalis.

The telencephalic floor, or subpallium, consists of several nuclei in addition to area superficialis basalis. Ventromedially, two distinct cell masses lie above the area superficialis basalis and the medial pallium (Figures 6, 7, 10, 15, 28). These nuclei probably represent the lateral and medial septal nuclei (ls, ms, Figures 6, 7, 10, 15, 28). In their topography and structure, they closely resemble similarly named nuclei in land vertebrates. However, nothing is known about their connections in elasmobranchs.

The ventrolateral telencephalic wall contains a number of cell groups I have tentatively labeled the striatum (st), based on their topography and the high acetylcholinesterase activity in sharks and batoids (unpublished observations). Among the chondrichthians I have examined, the topography of the striatum is clearest in *Notorynchus*. In this species, the striatum can first be recognized as a rapidly growing cellular ridge ventromedial to the lateral pallium (st, Figure 10A). As the ridge is traced caudally, it divides into distinct dorsal and ventral components.

The dorsal component is rapidly replaced by the expanding nucleus a (Figure 10B). However, the ventral component can be traced further caudally (Figure 10B, C), where it comes into close contact with, and may merge with, the forebrain bundles (Figure 10D). A similar striatal ridge can be recognized in *Squalus* (Figure 6A, C). In galeomorphs, however, the ventrolateral telencephalic wall is greatly thickened, and there is no trace of a

striatal ridge jutting into the lateral ventricle to mark the position of the striatum (Figure 6B, D). In *Mustelus* and *Triakis*, the same segment of the telencephalic wall is characterized by cell groups with high acetylcholinesterase activity—a reliable marker of the striatum in other vertebrates.

In neonate *Platyrhinoidis*, the ventrolateral telencephalic wall (Figure 7) is intermediate between that of *Squalus* and *Mustelus*. There is no distinct striatal ridge, but a similar cell group can be recognized lying ventromedial to the lateral pallium and adjacent to the lateral ventricle (st, Figure 7B, C). However, the telencephalon of adult *Platyrhinoidis* is more similar to that of *Raja*, and the lateral ventricles are reduced to approximately half their neonate volumes.

In *Raja* (Figure 15) and the more advanced batoids, the lateral ventricles are so reduced that the telencephalic hemispheres are essentially solid masses of neural tissue. In these taxa a chevron-shaped nucleus of larger clustered cells (st, Figure 15A, B, C) occupies the rostral half of the hemisphere and lies ventromedial to the lateral pallium and nucleus a as does the striatum in *Notorynchus* and *Squalus*, and is therefore a reasonable candidate for the batoid striatum. However, histochemical and experimental anatomical studies are needed to test hypotheses regarding striatal homologues in all elasmobranchs.

When summarized, the quest for a striatal homolog in chimaeras seems as fruitless as the scholastic question: How many angels can dance on the head of a pin. Kappers and Carpenter (1911) noted the strong, medially directed flexure of the caudal hemispheric wall in chimaeras particularly when the brain is viewed dorsally (Figure 8), and termed this caudal ridge the epistriatum, thus homologizing most of the telencephalic wall with the striatum of other vertebrates. Holmgren (1922) recognized both rostral and caudal striatal components in *Chimaera*, but noted he had no particular reason for doing so. His rostral component occupied the slight ventricular bulge of the lateral hemispheric wall, visible in Figure 28B, and the caudal component was defined as the bulk of the caudal hemispheric wall, visible in Figure 28C. Kuhlenbeck and Niimi (1969) reached conclusions similar to those of Kappers and Carpenter, and included cell groups w, x, and y of the present review in their striatal homolog. Faucette (1969a, 1969b) recognized three separate divisions of the striatum, essentially encompassing the lower half of the ventrolateral hemispheric wall (excluding area superficialis basalis) throughout the rostrocaudal extent of the telencephalon.

Analyses of the chimaerid lateral hemispheric wall are difficult, due to poor cellular differentiation in this area, and are further complicated by the caudally thickened unevaginated part of the wall (Figure 28C). At present, it is impossible to recognize a pallio-subpallial boundary for this part of the telencephalic wall. Therefore, its cell masses cannot be interpreted. Analysis of the chimaerid telencephalon will progress only by experimental study.

Finally, the telencephalon medium of elasmobranchs (Figures 6E, F, 7E, 10D, 15E) is formed by the ascending and descending forebrain bundles (fb), the preoptic area (pa), and the caudal part of nucleus a. Earlier in this section, I suggested that nucleus a might be the elasmobranch homolog of

the pallial amygdala in other vertebrates. This suggestion is based primarily on the relationships exhibited in *Notorynchus* (Figure 10). However, this region is more complicated in most other elasmobranchs, and this condition is likely due to a subdivision of nucleus a and/or the migration of additional pallial elements into this region in advanced elasmobranchs.

DISCUSSION

Brain—Behavioral Correlates

Fifteen years ago it was easy to characterize chondrichthians as primitive fishes with small, simple brains and limited behavioral repertoires. Renewed interest in all aspects of elasmobranch biology and the emergence of experimental neurobiological studies are forcing a reevaluation of these conclusions. Cartilaginous fishes possess large brains whose brain-to-body ratios fall within the ranges for birds and mammals. Their brains are not merely large; the relative development of their major divisions closely parallels that of birds and mammals (Bauchot et al. 1976, Ebbesson and Northcutt 1976, Northcutt 1977*b*).

The discovery that elasmobranchs possess high brain-to-body ratios was noted much earlier (Quiring 1941), but was reported as merely a curious finding that did not conform to Quiring's "scale of being" in which vertebrate brain size increased with supposed phylogenetic level. It would be equally fallacious simply to assume that elasmobranchs and mammals possessing comparable brain sizes also possess comparable neural capacities. We know too little about the neural basis of vertebrate behavior in general—and even less about that of elasmobranchs—to make such a bald assumption. However, one task of comparative neurobiology is to compare, and the number of rapidly accumulating similarities among large-brained vertebrates—restricted olfactory projections, differentiation of telencephalic pallial areas receiving multiple sensory inputs, long descending pallial efferents, and expanded cerebellar cortices—warrant comparisons in a search for possible common selective pressures that may underlie the evolution of large-brained vertebrates.

Pair bonding, parental care, endothermy, increased dependence on learned behaviors, a large number of species-specific behaviors, high levels of exploratory behavior, and extensive manipulation of food characterize birds and mammals. Clearly, these traits are not exclusive to birds and mammals, bony fishes and reptiles frequently exhibit one or more of these traits, but they are not seen in concert except in birds and mammals. However, there is a direct correlation between large brain size and complex behavior in bony fishes (Northcutt and Braford 1977) and reptiles (Platel 1976, Northcutt 1977*c*).

Pair bonding and parental care confer on offspring a number of advantages, including protection and extended maturation periods. These qualities, as such, are unknown in elasmobranchs (Gruber and Myrberg 1977). However, 22 of the 31 chondrichthian families are solely viviparous, with

gestation periods as long as two years (Wourms 1977). Representatives of four different families (carcharhinids, sphyrnids, dasyatids, and myliobatids) have evolved yolk sac placentas or placental analogs (trophonemata), increasing energy flow to the embryos by 800 to 5000 % (Wourms 1977). These same families have the most complex neural organization and the highest brain-to-body ratios known for elasmobranchs. Viviparity allows for larger offspring at birth, reducing the numbers of potential predators and competitors and increasing the number of potential prey items. Larger neonates also have greater locomotive and metabolic efficiency (Hutchinson and MacArthur 1959, Wourms 1977).

Endothermy in birds and mammals underlies their high activity levels, but it also imposes a high energy cost—some eightfold more than for ectotherms. The energy cost of endothermy demands that organisms using this strategy be far more efficient in obtaining food than their ectothermic competitors—perhaps one of the reasons for the correlation of large brain size with endothermy. There are few studies of elasmobranch temperature regulation and maintenance, but *Isurus* and *Lamna* are known to maintain body temperatures well above those of the ambient environment (Carey and Teal 1969). These species are high-speed predators feeding on equally fast-moving prey. We need information on the temperature regulatory abilities of other large-brained elasmobranchs—carcharhinids, sphyrnids, and myliobatiforms—to decide whether elevated body temperatures characterize large-brained elasmobranchs or reflect a specific adaptation of lamnid sharks.

We know virtually nothing about chondrichthian behavioral capacities. Long-term behavioral observations are almost impossible due to the difficulties of maintaining most species in captivity for any length of time. Field studies present equally formidable problems when the animals under study are swift, far-ranging species—many of whom are also efficient predators—in an environment alien to human observers (Nelson 1977). To date, the most detailed observations on the social behavior of chondrichthians are included in one study on bonnethead sharks (*Sphyrna tiburo*), carried out in a seminatural enclosure by Myrberg and Gruber (1974). These workers recognized 17 separate behavioral units, 8 of which occurred in a social context. Myrberg and Gruber's study does not include observations on courtship, mating, or feeding. Detailed observations on the behavioral repertoire of a single chondrichthian species do not exist, let alone the interspecific observations needed to recognize correlations and trends in brain-behavioral complexities.

While some learning studies have used sharks as subjects (see Gruber and Myrberg 1977 for a recent review), the tasks have been limited to simple Y-maze performance or brightness and pattern discrimination. These studies demonstrate that sharks learn at rates comparable to those of birds, mammals, and teleosts, but they tell us little about the role of learning in elasmobranch behavior or about the complexity of problems that elasmobranchs might handle. Evaluation of the role of learning in chondrichthians requires answers to questions that relate learning to the chondrichthian environment: do individuals learn to recognize one another in a social context, do they

learn to recognize potential predators, do they learn to identify and locate seasonally limited resources, and do they learn migratory routes?

Many birds and mammals exhibit extensive motor skills in the manipulation of food. Prolonged learning periods are frequently involved in mastering such skills as the oystercatchers' manipulation of mollusks and rodent and parrot manipulation of fruits and nuts. Considerable learning may be necessary for even recognition of food items. Comparable behaviors are unknown in elasmobranchs, but some aspects of feeding strategies appear to be correlated with brain size. Most squalomorph sharks, for example, have short jaws and feed on prey smaller than themselves by grasping or shearing the prey into smaller pieces. Many advanced galeomorph sharks possess a highly modified jaw apparatus that allows deep, gouge-like bites that are effective in attacks against prey larger than the predator (Moss 1977). Such an adaptation allows a predator to attack new prey species, but it also entails new types of risks to the predator. Large brain size in advanced galeomorph sharks could be correlated with increased sensory and motor abilities needed for successfully attacking large prey. It is also possible that learning plays an important role in modifying attack behavior.

In addition, the most successful large-brained sharks (carcharhinids) occur widely in reef communities, which are also the habitat of the largest-brained teleosts (Bauchot et al. 1977). The reef habitat is the most complex and stratified in the aquatic environment, and there would be selective advantage in predators' learning the complex spatial organization of the reef habitat, and recognizing and pursuing prey that is well-camouflaged or has complex defense mechanisms.

Our knowledge of chondrichthian biology is insufficient to allow accurate assessment of the neural capacities of these forms. However, the existing information is sufficient to discredit characterization of chondrichthians as creatures of limited behavioral abilities. The great variation in size and complexity of their central nervous systems argues strongly for a wide range of neural capabilities. Future studies may well reveal that advanced sharks and batoids possess many behaviors thought to be characteristic of only birds and mammals.

Persistent Central Nervous System Problems

Experimental data on chondrichthian CNS organization have accumulated rapidly in the last 10 years. However, the information is still rudimentary. No detailed quantitative data exist for chimaerids, thus it is impossible to compare them to elasmobranchs. In addition, analysis of their forebrain organization requires experimental data on olfactory and thalamic projections in chimaerids, as well as histochemical studies. It is possible that chimaerid forebrains retain a large number of ancestral characters common to early chondrichthians, but it is also possible that they possess pallial specializations acquired independently. This problem can be solved only by extensive experimental studies.

Preliminary data on spinal projections exist for two shark species— *Scyliorhinus caniculus* (Hayle 1973*a*) and *Ginglymostoma cirratum* (Ebbesson 1972). Similar data are lacking for batoids, and detailed studies are needed to determine the presence of dorsal column nuclei and their projections. At present, we possess no information on the organization of the trigeminal projections and central gustatory pathways.

Information on acousticolateralis organization is accumulating, but more detailed studies are needed to reveal whether ampullary, auditory, and ordinary neuromast systems possess separate pathways throughout the midbrain and forebrain.

In elasmobranchs, cerebellar size is clearly not correlated with body size in any simple way. It is not true that only small sharks possess smooth cerebella and only large sharks convoluted cerebella. Complex, convoluted cerebella characterize advanced galeomorph sharks and batoids, regardless of size. The cerebellum is traditionally believed to coordinate locomotion, but many vertebrates reveal complex sensory representations in the cerebellar cortex. Almost nothing is known about the sensory inputs or their segregation in elasmobranchs. Such data are needed to elucidate cerebellar development and the role of the cerebellum in integrating sensory modalities related to complex motor behaviors. Studies of sensory input are also most likely to explain why different portions of the cerebellar cortex (Figures 11, 14) have hypertrophied in galeomorph sharks and in advanced batoids.

Experimental studies have demonstrated that the optic tectum is a major visual center in sharks (Ebbesson and Ramsey 1968, Northcutt 1976), but Graeber and Ebbesson (1972*b*) have also shown that nurse sharks with extensive tectal ablations learn visual discrimination tasks. This result suggests similarities between elasmobranch and mammalian visual organization, and it clearly argues that other elasmobranch brain centers also mediate visual discriminations.

Cohen et al. (1973) revealed that visual information reaches the telencephalic pallium in sharks, but we still lack details on the visual pathway (or pathways) to that area. In land vertebrates, two or more visual pathways project upon the telencephalon, after thalamic synapse, forming retino-thalamo-telencephalic and retino-tecto-thalamo-telencephalic channels. We know that in sharks the thalamus receives both retinal and tectal input, but there are no anatomical data to indicate whether two visual pathways project from the thalamus to the telencephalon.

The vertebrate retina normally projects to several (8–10) primary neural populations, forming a large number of more or less distinct visual circuits with circumscribed functions. At present we possess some information on the anatomy of the chondrichthian retino-tecto-reticular circuit, and fragmentary information on the retino-thalamo-telencephalic circuit. We have essentially no information on the visual properties of single cells in the chondrichthian central nervous system. Thus, our data on the biology of chondrichthian vision is indeed fragmentary.

For the most part, information on the chondrichthian hypothalamus is limited to studies on the elasmobranch hypothalamo-pituitary axis

(Gorbman 1959, Wilson and Dodd, 1973), and inferior lobes (Evan et al. 1976, Demski 1977). We have no detailed information on the number of cell groups, their connections, and their integrative and control functions in chondrichthians. Such data are clearly needed if we hope to make any headway in understanding chondrichthian feeding, reproductive, and homeostatic functions.

Detailed studies are also needed for the other two diencephalic regions, epithalamus and thalamus. The habenular nuclei form part of a sizable descending brain stem pathway, via the fasciculus retroflexus, but little is known about the sources of input to the habenula. The habenular region is likely a focal point of epiphyseal, hypothalamic, telencephalic, and raphe inputs involved in one or more biological rhythms, but the nature of these pathways and their functions are unknown. Spinal, cerebellar, tectal, and telencephalic inputs to the elasmobranch thalamus have been demonstrated experimentally, as have projections to the telencephalon. However, there are no detailed studies on the cytoarchitecture of the thalamus or its variation. Until we have data on the number of thalamic nuclei, their connections, and their physiology, it is impossible to compare chondrichthian thalamic evolution and organization to that of other vertebrates in any detail.

One of the most striking elasmobranch neural trends is the elaboration of the telencephalic central nucleus (Figures 6, 7, 10, 15). The central nucleus bears certain topographic and embryonic similarities to the expanded pallium of actinopterygian fishes (Northcutt and Braford 1977), to the dorsal ventricular ridge of sauropsid reptiles (Cohen and Karten 1974, Northcutt 1977c), and to parts of the mammalian isocortex (Karten 1969, Northcutt 1969a, 1969b). All of these telencephalic structures arise from dorsal and/or lateral telencephalic pallial fields, and all receive various sensory inputs from the thalamus. In land vertebrates, each modality is represented in a restricted portion of the hypertrophied pallium, and extensive efferents project to the striatum. Physiological studies (Cohen et al. 1973, Platt et al. 1974) indicate that the elasmobranch central nucleus is probably organized in a similar manner. However, anatomical details on the distribution and number of ascending sensory projections to the central nucleus are lacking, as are data on the pattern of efferent projections from this nucleus.

The elasmobranch central nucleus is a tempting potential homolog to the diverse pallial specializations of other vertebrates. It is far more likely, however, that the elasmobranch pallial condition has evolved independently, in parallel with the pallia of advanced actinopterygian fishes and amniotic vertebrates.

Comparison of elasmobranch brain evolution with that of other vertebrate groups reveals a number of similarities: increase in brain size, restricted olfactory projections to the telencephalon, expansion of the striatum, and expansion and differentiation of the nonolfactory telencephalic pallium. Similar trends of independent origin occur in teleosts (Northcutt and Braford 1977), reptiles (Northcutt 1977c), birds (Stingelin 1958), and mammals (Jerison 1973, Welker 1976). Undoubtedly there are inherent constraints on neuronal evolution, and finite possibilities for change in neural

populations. Given these restrictions, it is likely that specific solutions to specific problems have been "discovered" independently by different verte-brates in the course of their evolution. Our understanding of these problems and their neural solutions require continued study of the neurobiology, be-havior, and natural history of chondrichthians and other vertebrates.

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BEHAVIORAL STUDIES CORRELATED WITH CENTRAL NERVOUS

SYSTEM INTEGRATION OF VISION IN SHARKS

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BRAIN—BEHAVIOR RELATIONSHIPS

A Systematic Approach to Shark Behavior

The design of an animal's central nervous sytem is highly correlated with the characteristics of its sensory input and behavioral output. The specific strengths and limitations of these two variables are reflected in the structure and organization of the brain that processes them. Thus, information regarding the sensory and behavioral capabilities of a particular species or class of animals can be a helpful guide to its brain function. Similarly, detailed knowledge about an animal's brain structure can provide clues to its general behavioral repertoire.

Aronson (1963), in his review of the elasmobranch central nervous system, underscored the potential usefulness of this inferential approach, suggesting that it would hasten our understanding of shark behavior by providing a theoretical framework for experimentation. Several years later Masai (1969) became the first investigator to heed Aronson's advice. Starting with gross neuroanatomical descriptions of 12 Pacific and Indian Ocean shark species, he demonstrated correlations between their external brain morphology and known behavioral characteristics. Masai's macroanalysis was limited, however, by the generality of the neuroanatomical and behavioral data available to him, and it must therefore be considered more intriguing than useful for precise prediction of either behavior or morphology.

The opportunity now exists for a much more detailed and productive analysis. The recent resurgence of elasmobranch research has produced a wealth of sensory and behavioral information about sharks. In general, the recent findings of sensory biology indicate that their sensory systems are remarkably diverse and sensitive, compared to those of most other extant animal species. For instance, we now know that a great many sharks have a duplex retina, which is at least as sensitive as man's and can detect light from all parts of our visible spectrum (Gruber 1975). Kalmijn (1966) has demonstrated the presence of electroreceptors that are probably the most sensitive in the animal kingdom. Similar examples of sharks' unusual sensory capabilities can be found throughout this book.

From a behavioral perspective, other studies have shown that sharks can modify their responses in reaction to specific environmental stimuli. Although the precise extent of this behavioral flexibility has not yet been defined, substantial evidence indicates that sharks can learn certain types of instrumental discrimination tasks as rapidly as most mammals (Aronson et al. 1967, Graeber 1972, Graeber and Ebbesson 1972a, Kelly and Nelson

1975, Kritzler and Wood 1961, Nelson 1967). They also respond quickly to standard classical conditioning procedures (Gruber and Schneiderman 1975).

Similar breakthroughs have been made in the study of shark neuroanatomy (see Northcutt 1978). As a result, the potential now exists for integrating these related sets of data into a tentative model of the shark brain and behavior relationship. While the purpose of this chapter is not to construct a model, its contents are offered in support of such a project's feasibility. As Aronson (1963) suggested over 10 years ago, the marriange of behavioral analysis and experimental neuroanatomy is long overdue in shark laboratories. Such a model, if developed, would provide needed insights into the functioning of central neural systems now poorly understood in sharks. It might also heighten our understanding of the behavior of pelagic and other shark species that are poorly suited for laboratory study.

Traditional Shark Neurology

Although the recent findings of sensory and behavioral biology are encouraging, they raise some difficult questions about brain structure when contrasted with the classic views of comparative neurology. The major problem is the apparent paradox of a complex set of sensory inputs and behavioral outputs being processed by a relatively simple, undifferentiated central nervous system.

The concept of the shark brain as primitive and dominated by olfactory areas dates back to the early twentieth century. Comparative neurologists, attempting to reconstruct theoretically the evolution of the vertebrate brain, quickly recognized the importance of sharks as "living fossils" and began studying them intensively. Their findings reflected the prevailing views of biologists regarding shark sensory capabilities, particularly those of Parker and Sheldon who had elegantly demonstrated the dogfish's (*Mustelus canis*) apparent total dependence on smell for locating prey (Parker 1910, Parker and Sheldon 1913, Sheldon 1911). Why else was the telencephalon occupied almost entirely by olfactory areas and pathways? Meanwhile, the functional roles of other known senses, such as vision, appeared to be secondary and less demanding of high-level central integrating mechanisms. The anatomical restriction of visual input to the midbrain optic tectum supported this notion, especially considering the lack of tectal differentiation in sharks as compared to teleosts.

Even today many authors (Aronson 1963, Lineaweaver and Backus 1973, Nieuwenhuys 1967) state that the shark brain consists of poorly interrelated areas devoted mainly to sensation, especially olfaction. They note that the forebrain appears to lack distinct nuclear groups and to be devoid of any neural basis for associative processes (Voronin et al. 1968), a finding consistent with the view that sharks are primitive fish whose behavior is often "clumsy" and is based mainly on instinctive stereotyped response patterns (Aronson 1963). It is therefore not surprising that no attempt has been made to search for a neural substrate underlying behavioral flexibility in these fish.

Recent Neuroanatomical Findings

As recently as a decade ago there was little reason to question the traditional view of shark neuroanatomy based on studies of normal shark brains. However, the situation suddenly changed when anatomists developed ways to modify modern mammalian histological techniques to study neural degeneration in poikilotherms (Ebbesson 1970). The new findings, as described in this volume, have revolutionized our understanding of the structure and organization of the shark brain. As a result, it is now known that only about 10% of the telencephalon is devoted to olfaction (Ebbesson 1972a, Ebbesson and Heimer 1970). The functions of the remaining 90% are still unclear, but there is no doubt that other sensory inputs are present (Cohen, Duff, and Ebbesson 1973, Veselkin and Kovacevic 1973). Already extensive connections have been documented between the telencephalon and the thalamus, optic tectum, and brainstem nuclei (Schroeder and Ebbesson 1974). At the very least, these findings suggest that the neural basis of shark behavior is much more complex than previously imagined.

Unfortunately, understanding the functional organization of the shark brain requires much more than a strictly anatomical blueprint of connecting areas and pathways. Aronson recognized this need in his 1963 review of the older neuroanatomical literature by pointing out that the behavioral evidence used to interpret morphological findings was based mostly on unreliable casual observations. It should now be apparent that only by a combination of electrophysiological and systematic behavioral techniques will we be able to determine what information these pathways transmit and how their terminal areas interact. It is tempting to speculate that had these functional techniques been more enthusiastically applied to sharks in the past, as they were to mammals, it might not have taken over 50 years to reveal the errors of traditional neuroanatomy. Yet the fruitfulness of the functional approach has been recognized only recently.

A series of studies concerning the shark central visual system has challenged the classic notion of visual processing being under exclusive tectal control. Although electrophysiological data helped to clarify the functional anatomy of this system, the emphasis in this chapter will be on the complementary approach to functional analysis: the use of selective neurological damage to experimentally manipulate visually mediated behavior.

BEHAVIORAL ASSESSMENT OF VISUAL FUNCTION

A variety of behavioral techniques have been used successfully since Lashley's time to examine postoperative visual losses in mammals. The simplest of these was first applied to sharks at the turn of the century, but investigators have since been reluctant to employ other, more sophisticated, assessment techniques. Much of the delay has resulted from the common view that active sharks are ill suited to controlled laboratory research conditions and are unable to learn standard behavioral tasks. A general consideration of the

feasibility and advantages of these techniques should help place current findings in their proper perspective.

Unlearned or Natural Responses

In the literature on mammals, the testing of unlearned, or natural, responses typically involves the postoperative study of visual following or avoidance. While these may involve only eye movement, they more often include movement of the limbs or of the entire body. Regardless of the exact nature of the chosen response, this type of visual assessment has several advantages that make it potentially valuable for use with sharks.

A primary consideration is the "built-in" nature of the response, which allows highly repeatable and easily elicitable behaviors to be monitored on a continuing basis almost immediately after completion of surgery. This permits greater flexibility in research design and yet requires less of the investigator's time per animal than usually demanded by rigorous training procedures. It also reduces the total amount of time before and after surgery that the experimental animal must spend captive in a laboratory setting. In planning shark research this time can be an important health and cost-related consideration (Clark 1963). Moreover, the technology for stimulating and observing unlearned responses is typically simpler than that for testing learned responses. This makes it more feasible to conduct shark neurological studies in less formal laboratory settings, reducing cost and allowing for a more realistic, natural test situation. The latter consideration assumes particular importance in the study of sharks because of the real-life hazards they pose and the need for data to help reduce their threat. In this respect the investigator can benefit by studying experimentally induced changes in an animal's normal behavioral repertoire, rather than studying changes in learned behavior patterns. Interpretive pitfalls are less likely, and findings can be more meaningfully related to behavior outside the laboratory.

Despite the potential advantages of testing unlearned visually guided, or elicited, responses after surgery, only a few investigators have done so. Steiner (1886) was the first to carry out nonsystematic general observations of swimming behavior following selective ablation of the telencephalon, diencephalon, or mesencephalon of sharks (*Scyliorhinus canicula*). Similar experiments were subsequently conducted by Loeb (1891), Bethe (1899), and Polimanti (1911, 1913). They reported on the fish's postoperative ability to maintain normal balance and to move spontaneously in the aquarium without bumping into the sides. Rizzolo (1929) extended these observations to *Galeus canis* and showed that forebrain removal did not disturb righting ability or posture while resting. Except for two studies concerning the role of the tectum in maintaining balance (Rizzolo 1929, Ten Cate 1931), there has been no subsequent use of purely observational techniques for assessing the consequences of damage to the shark forebrain or mesencephalon. Although all these early reports are limited by the sole use of dogfish, their findings will be discussed later in relation to other, more recent, findings about the functions of these two areas.

Considering the rapidly growing interest in shark ethology (e.g., Allee and Dickinson 1954, Myrberg and Gruber 1974), it is conceivable that more detailed information will soon become available about the natural response patterns sharks exhibit in reaction to specific visual stimuli. The work of Nelson and his colleagues (e.g., Johnson and Nelson 1973) demonstrates, on a relatively complex level, the potential contribution of this type of behavioral analysis to future neurological studies. Studying the gray reef shark (*Carcharhinus menisorrah*) in its natural surroundings, they have demonstrated the importance of visual cues in eliciting a well-defined stereotyped preattack behavior pattern in response to a diver's presence. By systematically varying the relative position of diver to shark, they have also been able to control the intensity of the agonistic display. It is not unreasonable to assume that appropriate neurological studies of sharks in semicaptivity could now be conducted to determine what central neural mechanisms process the pertinent sensory information, visual or otherwise, and trigger the behavior.

Likewise, Myrberg and Gruber (1974) have reported the triggering of less complex, modal action patterns in captive young bonnethead sharks (*Sphyrna tiburo*). They state that visual cues again appeared important, eliciting stereotyped responses to companion sharks and other environmental stimuli. Other studies, using chemical stimuli, indicate that once certain neural mechanisms of behavior are triggered, sharks will automatically carry out the corresponding behavioral response pattern to completion, even in the absence of the initial stimulus (Hodgson and Mathewson, 1971; Hodgson, Mathewson, and Carsten, personal communication). Unfortunately, the central neural mechanisms responsible for these induced behavior patterns have not yet been identified. Demski's (1977) recent attempt to apply brain stimulation to freely swimming sharks is a significant step toward providing such information. It is likely that other visually controlled innate response patterns await discovery and, hopefully, subsequent exploitation by neurophysiological and neurophychological analysis.

Learned Responses

Classical Conditioning—In the mammalian literature, classical, or respondent, conditioning is often used to determine whether an animal suffering a neural loss can detect various types or characteristics of visual stimulation. In the typical conditioning paradigm the animal is either restrained or partially paralyzed while receiving a conditioning stimulus paired with a mild shock to produce an eyeblink response or a change in heart rate. The passive nature of the test situation enables experimental separation of sensory losses from motor-related losses that might otherwise impair the animal's ability to translate perceived visual information into appropriate voluntary instrumental behavior patterns. Training can usually be accomplished relatively quickly, while permitting very precise control over stimulus and response characteristics. Despite these advantages, classical conditioning procedures require substantial laboratory equipment and are limited to pro-

viding information about conditionable stimuli (e.g., hue, flicker, frequency, and brightness) which can be differentiated by an unoperated animal during the few seconds, or less, of stimulus presentation.

A survey of the literature reveals that there have been only a few recorded attempts to classically condition sharks. Karamian (1956) described the first such experiment, carried out by Baru on dogfish in his laboratory. She obtained rapid formation of conditioned responses to light onset, or the sound of a bell, paired with shock; however, in comparison to similar conditioning in teleosts, the shark responses were characteristically unstable. The possibility exists that they may have resulted from improperly controlled nonassociative factors, such as sensitization or pseudo-conditioning, but sufficient procedural details from which to judge are not available.

The first definitive report on classical conditioning in sharks was recently published by Gruber and Schneiderman (1975). Using restrained young lemon sharks (*Negaprion brevirostris*), they demonstrated successful conditioning of the nictitating membrane response (eye blinking) to a light flash. All animals were conditioned within the first 100-trial session, and, by the second daily session, their performance had stabilized at the 95% level of conditioned responding. Appropriate controls ruled out any possible influence of nonassociative factors. The authors emphasize that the response characteristics resembled those seen in mammals, during both acquisition and extinction. This similarity suggests the future use of conditioning as a tool for comparing sharks with other groups of animals and for studying elasmobranch neurobiological mechanisms, especially central visual processing.

Instrumental Conditioning—Although vision's basic function is to detect photic stimuli, its role is more typically thought to include the guiding of instrumental behavior. Consequently, many investigators employ instrumental conditioning procedures to study the functional contributions of various central visual system components. Some use traditional training techniques whereby animals are administered a fixed number of trials per day at a predetermined rate, while others use more recently developed operant conditioning methods. In contrast to classical conditioning, both techniques permit the viewing of fairly complex visual stimuli that can be varied according to the timing, patterning, or directionality of light presentation.

Because learning does not depend on the precise timing of stimulation, the animal has sufficient opportunity to extract relevant stimulus information. By varying response requirements one can also examine how perceived visual information is translated into appropriate action patterns (Schneider 1969). Thus, the investigator can examine responsiveness to a greater range of visual stimuli than is possible using classical conditioning or natural response testing and can obtain a more comprehensive view of surgically induced deficits.

Here again, progress has been hindered by the common view that sharks are primarily creatures of primitive instinct, practically impossible to train.

Clark (1959) was the first to argue against this belief by demonstrating that sharks can be instrumentally conditioned to respond to visual cues (i.e., bump a target). Several subsequent studies (Aronson et al. 1967, Clark 1961, Graeber and Ebbesson 1972a, Tester and Kato 1966) have confirmed her basic finding and have extended the results to include discrimination learning. However, caution should be exercised in interpreting some of these later reports. In particular, Clark (1961) trained lemon sharks on color and shape discrimination tasks but did not control adequately for brightness differences, left—right target positions, or transfer effects among the five target pairs. Aronson and his colleagues (1967) reported some intriguing results based on operant light—dark discrimination, but they tested only one animal. Nevertheless, the feasibility of using instrumental visual discrimination as a tool for the neurological testing of elasmobranchs has been adequately demonstrated. Sharks can at least learn light—dark and simple form discriminations, including upright vs inverted triangles (Graeber 1972), without much difficulty. Whether they can also learn to discriminate hue and more complex visual patterns remains to be seen.

MIDBRAIN VISUAL MECHANISMS

The "Dominant" Tectum

The mesencephalic optic tectum traditionally has been considered the predominant, if not sole, recipient of retinal afferents in both teleosts and elasmobranchs. Consequently, it is often referred to as "the visual center" for all fish (Aronson 1963, Healey 1957, Ten Cate 1935).

The results of previous behavioral studies supported this concept and led Aronson (1963, p. 205) to state that "Most investigators agree that removal of the optic lobes causes blindness in both elasmobranchs and teleosts." Upon closer examination, however, the empirical evidence for sharks is extremely limited compared to that for teleosts. Steiner (1888, cited by Ten Cate 1935), using general observations, was the first to report that blindness follows removal of the optic lobes in sharks. Since then only Polimanti (1913) and Rizzolo (1929) have reported similar results.

The behavior Polimanti attributed to blindness in *Scyllium* may not have resulted from the removal of the tectal visual center, but rather from the severe oculomotor, locomotor (forced circling), and pupillary deficits, which he also reported. The latter effects suggest that the surgical damage extended below the tectum into the underlying tegmentum. The most complete description of blindness is that of Rizzolo, who stated that subjects (*Galeus canis*) with bilateral optic lobe ablations failed to avoid the sides of the aquarium. The validity of his conclusion is also questionable, however, in that all of the operated sharks died within two or three days after the operation. This suggests that the "blindness" was due not to the tectal lesions per se but to other factors, such as possible ionic imbalance caused by incomplete protection of the wound.

In addition to the problem of interpreting previous behavioral experiments there have been enough substantial changes in our understanding of shark neuroanatomy to warrant reexamining the behavioral effects of tectal lesions. Because of evidence from selective silver impregnation studies, we now know that the shark central visual system extends far beyond the optic tectum. In the three species so far examined (*Ginglymostoma cirratum*, *Galeocerdo cuvieri*, and *Negaprion brevirostris*), retinal fibers project to the dorsolateral thalamus, pretectal area, and hypothalamus in addition to the traditionally recognized tectum (Ebbesson and Ramsey 1968, Graeber and Ebbesson 1972*b*). The extent of these diencephalic visual projections suggests that the optic tectum may not exert exclusive control over the visuomotor behavior of elasmobranchs. Other areas of the brain may be equally important in processing certain types of visual information.

Tectal Ablation and Visual Discrimination

As in past research, our work has focused on the importance of the optic tectum in guiding behavior. In view of the success of Clark and her colleagues, we chose a relatively simple instrumental visual discrimination task for the animals to learn. Although Clark had not attempted to train isolated individual sharks on this type of task, it appeared ideal because it involves an active organism but still provides relatively rigorous control over experimental conditions. Also, in contrast to operant procedures, the fixed-trial instrumental method allows response latency to be measured, thus providing an index of possible motivational deficits resulting from nonspecific surgical trauma. The general method and results of this study are described below, but the interested reader may wish to consult the two previously published reports for further details (Graeber and Ebbesson 1972*a*, Graeber, Ebbesson, and Jane 1973).

Juvenile nurse sharks, 2 to 4 ft long, were chosen as subjects because of the species' previous use in both anatomical and conditioning studies, well-documented hardiness in captivity, and ready availability. Prior to receiving any training, they underwent bilateral aspiration of the optic tectum while anesthetized in a 0.01% bath of tricaine methane-sulfonate and seawater. Care was taken to limit the ventral extent of the lesions to that region of the tectum known to receive retinal input. The brain case hole was then closed with dental cement, and the skin incision sutured with silk or stainless steel wire.

After surgery the sharks were moved into one of two maintenance pens (see Figure 1) where they remained until they began to eat well (9 to 27 days). As each one recovered, it was deprived of food for 48 h before being adapted to the apparatus and taught to swim in a clockwise direction down the test alley, through the goal area, and back up a corridor to the starting pen. Other sharks remained unoperated and served as controls.

Actual discrimination training began when they were required to choose the correct target door in a modified-Y maze. Each shark was given six consecutive trials daily, about 45 s apart, and was rewarded each time with a
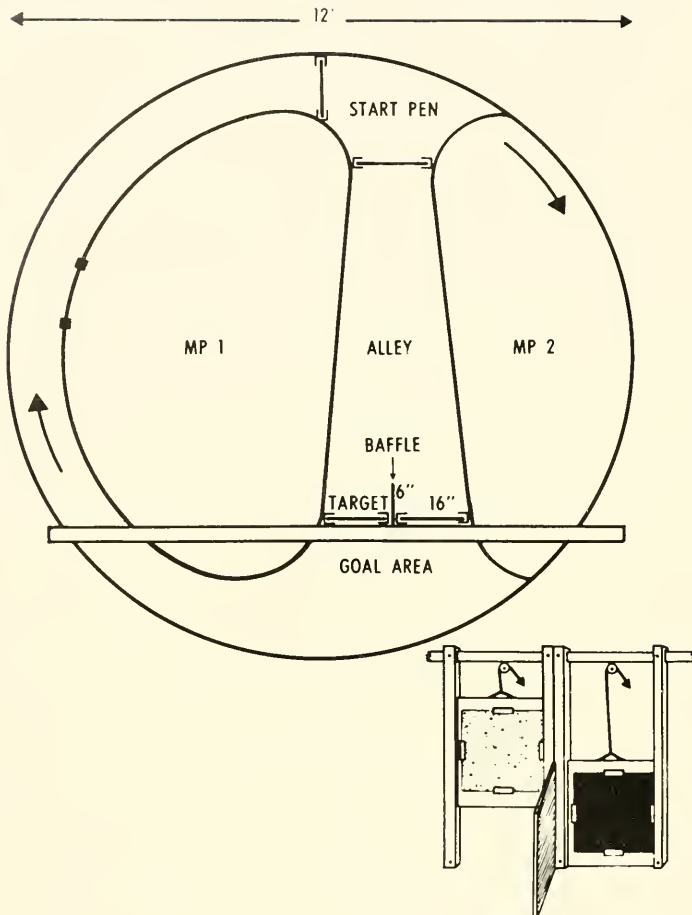
Figure 1   Schematic of conditioning pool divided by sheets of
black Plexiglas into maintenance pens (MP) and a modified-Y
maze. The 3-ft-high pool was filled to a depth of 20 in. and was
shaded by a 6-ft-high tarp roof. The target doors (lower right)
were built of transparent Plexiglas with mountings to hold inter-
changeable square targets.

standard piece of thawed mackerel. Care was taken to prevent any olfactory
cues from biasing the shark's response tendencies by presenting the fish
reward impaled on a stiff wire only after the shark had entered the goal area.
If a subject did not choose the correct target within 60 s after beginning a
trial, it was guided through the correct door, but an error was recorded. The
discriminations were considered learned ($p < 0.01$) when the shark made at
least seventeen correct responses over three consecutive days (Bogartz 1965).

The sharks were trained first on a black-vs-white discrimination and then
on a horizontal-vs-vertical pattern discrimination in which each 30.4-cm^2
target was painted with three black and three white 2-in-wide stripes. Each
of these striped targets served as correct or incorrect an equal number of

times daily, to control for cues other than stripe orientation that might be associated with a particular target. To rule out position learning for both tasks, the left-right location of the correct target was varied quasi-randomly in a predetermined fashion. A 15.2-cm-long opaque baffle separated the two targets and forced the sharks to choose one or the other before swimming across a choice line drawn even with the baffle's front edge. The experimenter observed the responses in a mirror suspended from the roof of the pool and opened the door when the correct target was chosen.

Contrary to the findings of previous investigations, removal of the optic tectum did not prevent the sharks' learning visual discriminations. They all learned to discriminate black from white, and only one failed to reach criterion on the horizontal-vs-vertical stripes discrimination. This failure is likely due to the lack of time to train it for more than half the number of trials permitted the others.

In comparing the performances of the operated sharks with those of the unoperated controls (Table 1), we find little evidence to suggest any substantial postoperative visual discrimination deficit. In fact, some of the tectally ablated sharks actually learned to discriminate faster and with fewer errors than did their unoperated counterparts. The learning curves of one of the operated subjects is shown in Figure 2. The apparently lengthy training required by NS-184 on the black—white task is misleading in that the shark performed significantly above chance (p < 0.005) after only 107 trials and 41 errors (Grant 1947). It continued to perform at this level for the next 10 sessions, after which training had to be discontinued for 16 days for unrelated reasons. The lack of a surgically related performance deficit is also reflected in the median daily response latencies for both groups, which quickly decreased to a stable level (5-10 s) after a few days of training.

Table 1. Number of trials and errors to criterion on two visual discrimination tasks for sharks with and without tectal ablations

Subject	Black-white		HV Stripes	
	Trials	Errors	Trials	Errors
Operated				
NS-143	203	72	213	75
NS-184	378*	109	(114)†	(51)†
NS-187	318	106	264	112
Unoperated				
NS-144	96	25	48	9
NS-146	183	49	219	78
NS-147	108	38	186	63

*See text for explanation.
†Parentheses indicate that shark did not reach criterion.
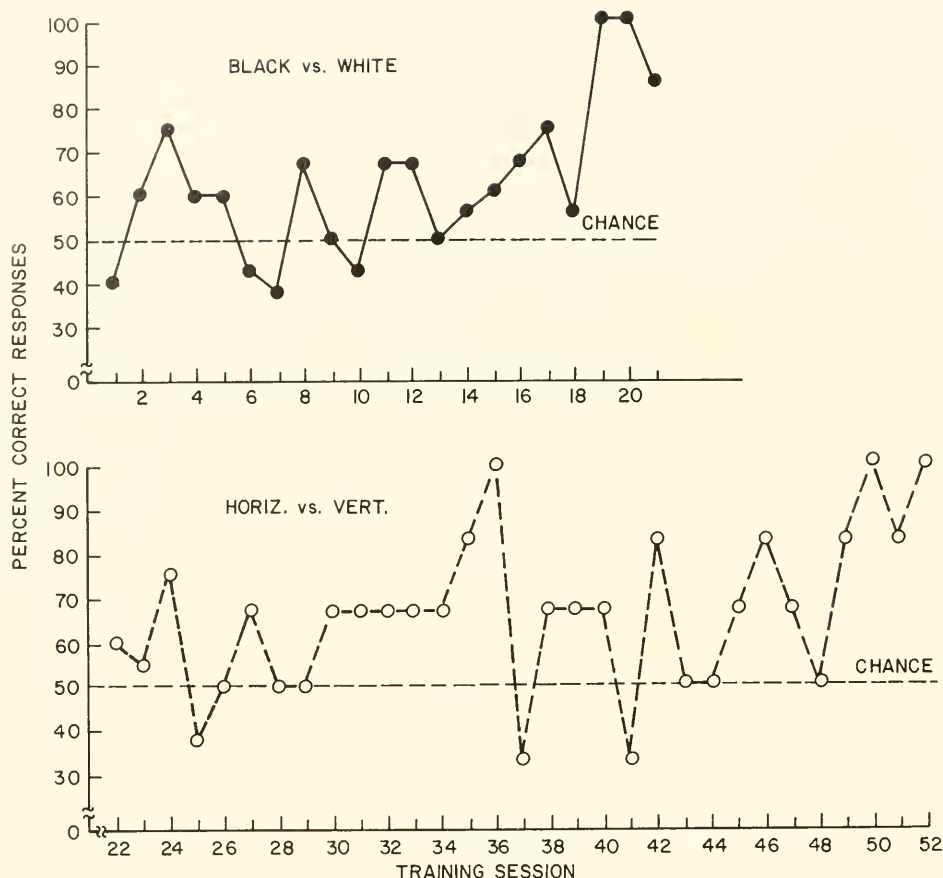
Figure 2   Daily performance, after surgery, of tectal-lesioned shark NS-143 on the tasks of discriminating black and white and horizontal and vertical stripes.

After training, the operated sharks were sacrificed by intracardiac perfu-sion with 10% formalin. After removal, the brains were frozen and sectioned transversely every 50 μm. Every twelfth section was subsequently stained for cell bodies with cresyl-violet (Ebbesson 1970). The extent of ablations in one subject is shown in Figure 3. In the other sharks the optic tectum was also totally removed except for about 5% of the lateral portion of subject 187's right tectum. The lesions completely abolished both the upper and lower tectal layers, which respectively receive the retinal and telencephalic input. There was little, if any, damage to adjacent regions of the brain, in-cluding the underlying tegmentum, except for caudal portions of the pretectal area in NS-184.

Despite the similarities in discrimination task performance between the operated and unoperated sharks, one might suspect that removing such a substantial amount of central visual neuropil must have disrupted visual

NORMAL   OPERATED

Figure 3   Nissl-stained sections cut transversely, 600 μm apart, through the midbrains of a normal nurse shark (left) and NS-143 (right), indicating a complete tectal ablation in the latter. Sections are arranged rostrocaudally (A-D) with the following abbreviations: TeO, optic tectum; AP, pretectal area; GL, lateral geniculate nucleus (dorsolateral optic nucleus); CTh, central thalamic nucleus.

function somewhat. This is further suggested by the general behavior of subject NS-143, observed during the recovery period. At first the shark remained relatively inactive, lying on the bottom of the pool and occasionally

bumping into surrounding walls and objects when attempting to swim. After a few days it ceased colliding, but, in spite of its great readiness to eat, remained extremely reluctant to move forward in any direction, even for food. Upon beginning pretraining 27 days after surgery, the shark swam sluggishly, required frequent coaxing and prodding, and often hesitated before entering the goal area beyond the target doors. After a day or two these behaviors disappeared; the shark began to swim readily and successfully completed pretraining within a total of 9 days.

The behavior of NS-143 may indicate that successful discriminative learning represents a recovery of visual function mediated by some other area of the central visual system. However, general observation of the other tectal subjects revealed no evidence of initial postoperative blindness. In fact, one began successful pretraining only 9 days after surgery. The question of recovery of function will not be answered until the completion of experiments examining the effects of tectal lesions on the retention of preoperatively learned visual discriminations. Yet, the following evidence for telencephalic involvement in shark vision offers a likely candidate for the recovery mechanism, if one is necessary.

The present results allow us at least to conclude that the optic tectum is not necessary for visually guided behavior in the nurse shark. The similarity of the retinal projections in this species to those in the two other species so far examined, *Negaprion brevirostris* and *Galeocerdo cuvieri*, further increases the possibility that the tectum is not vital to the performance of such behavior in most sharks. Speculation on the true role of the midbrain in shark vision would be better left until after a review of forebrain involvement.

FOREBRAIN VISUAL MECHANISMS

Do They Exist?

Neuroanatomical Considerations—The extensive studies of early comparative neuroanatomists (Ariens Kappers 1906, Bäckström 1924, Herrick 1922, Houser, 1901, Johnston 1911) strongly supported the common belief (Aronson 1963, Nieuwenhuys 1967) that the elasmobranch telencephalon serves only as an olfactory-gustatory coordination center. These observations were based on the examination of "normal" material from nonlesioned animals. Because the shark brain is characterized by many thin and poorly myelinated axons connecting diffuse cell groups, it is very difficult to distinguish its structural organization even under today's light microscopes.

In the 1950s Nauta developed the selective silver impregnation technique to trace degenerating neural pathways (Nauta 1957, Nauta and Gygax 1954). This technological breakthrough, coupled with Fink and Heimer's (1967) new method for staining degenerating axon terminals, led the way to a host of new discoveries about the structure of the mammalian brain. Similarly, Ebbesson's (1970) subsequent modifications of the Nauta-Gygax and Fink-Heimer histological techniques for use in fish provided the first opportunity

to reexamine the conclusions of earlier workers with greater accuracy and much more detail.

The predominance of olfaction in the shark telencephalon was first questioned by the finding that the bulk of the nurse shark's telencephalon does not receive any primary or higher order olfactory input (Ebbesson 1972a, Ebbesson and Heimer 1970). The possibility of visual information reaching the telencephalon was immediately suggested by the previously unknown presence of extensive retinal projections to the dorsolateral thalamus of lemon, nurse, and tiger sharks (Ebbesson and Ramsey 1968, Graeber and Ebbesson 1972b). The other major recipient of retinal fibers, the optic tectum, has also been shown to send a massive ascending projection to this same region of the thalamus (Ebbesson 1971, 1972b). When experimental lesions are made in this dorsolateral area, degenerating axons are found to project upward in a sizeable pathway to the contralateral central telencephalic nucleus (Ebbesson and Schroeder 1971, Schroeder and Ebbesson 1974).

Anatomically this thalamo-telencephalic pathway, though crossed, is reminiscent of the classic geniculo-striate visual connection in mammals. However, the inclusion of tectal input in the thalamic area of origin implies that this tract in sharks may also relay visual information derived from the tectum. In mammals, the latter function is performed by a second, separate pathway connecting the tectum to the lateral posterior, or pulvinar, nucleus of the thalamus and then ascending to terminate in the extra-striate visual areas of the cortex. The substantial overlap between retinal and tectal terminations in the shark thalamus suggests that perhaps the two separate ascending systems of mammals may be combined in these fish in a manner closely resembling the central visual system of some common vertebrate ancestor (Ebbesson 1972b).

Telencephalic Visual Evoked Potentials—Further support for telencephalic visual function in elasmobranchs is provided by reports of evoked potentials being recorded in response to electrical stimulation of the optic nerves in nurse sharks (Cohen et al. 1973) and rays (Veselkin and Kovacevic 1973). Cohen and his colleagues obtained short-latency field potentials localized in the posterior portion of the ipsilateral, central telencephalic nucleus after stimulating the optic nerve with monophasic square waves 0.1 ms in duration (Figure 4). In confirmation of the morphological data, they noted that the physiologically responsive area overlaps the area known to receive fibers from the thalamic visual region. The lack of evoked potentials in the contralateral telencephalon also substantiates the total recrossing of the thalamo-telencephalic visual pathway after complete decussation of the retinal fibers at the optic chiasm.

Veselkin and Kovacevic (1973) examined the presence of visual evoked potentials in the telencephalons of the rays *Dasyatis pastinaca*, *Raja clavata*, and *Torpedo ocellata* and of the shark *Scyllium cannicula*. They also found ipsilateral responses to optic nerve stimulation in all three species of rays, but not in *Scyllium*. These responses were localized in the posterior telen-
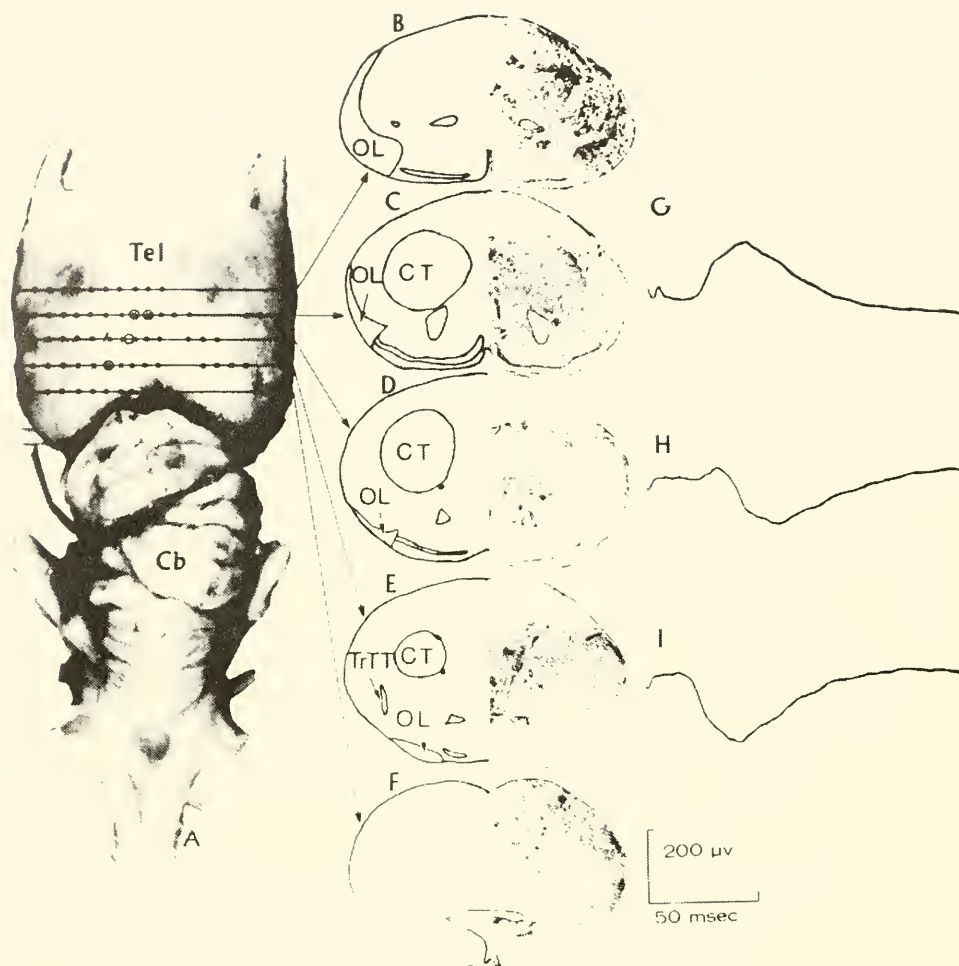
Figure 4 (A) Dorsal view of the nurse shark brain, with schematic of the sites of electrode penetrations in the telencephalon (Tel). The horizontal lines are separated by 2 mm, and each symbol indicates a penetration site. Mediolateral symbol separations are equivalent to 1 mm. The crosses on the ipsilateral (left) telencephalon indicate penetrations where field potentials were evoked in response to optic nerve stimulation. The large open circles indicate penetrations where maximum activity was obtained. The cerebellum is labeled Cb. (B-F) Transverse sections through telencephalic levels indicated by leaders from (A). The right half of each section is stained with cresylechtviolett to illustrate the prominent central telencephalic nucleus (CT). The other abbreviations are OL, lateral olfactory area; TrTT, thalamotelencephalic tract. (G-I) Representative waves elicited in penetrations at levels corresponding to (C), (D), and (E), respectively. Downward deflections indicate negativity. (Reprinted with permission from Cohen et al., *Science* 182:492-494 (Nov. 2, 1973), Fig. 1. Copyright 1967 by the American Association for the Advancement of Science.).

cephalon, slightly more medially than those found by Cohen et al. in the nurse shark and as predicted by the anatomical findings of Ebbesson and Schroeder (1971). The authors conclude that the absence of telencephalic visual responses in *Scyllium* is due to functional, not morphological, differences since they simultaneously confirmed the presence of the crossed thalamo-telencephalic pathway by recording evoked potentials in response to stimulation of the contralateral dorsal thalamus.

Past Behavioral Studies--In accordance with the early anatomical literature, the belief that the telencephalon plays no role in shark vision is fairly well supported by the findings of previous behavioral studies (Healey 1957). All investigators who have examined the effects of telencephalic ablation have reported that there are no postoperative disorders in vision, locomotion, or equilibrium (Bethe 1899, Karamian 1956, Loeb 1891, Polimanti 1911, 1913, Rizzolo 1929, Steiner 1886, 1888). Thus, Aronson (1963, p. 220) has stated that "on the basis of general observations in an aquarium, it is hardly possible to distinguish an operated from an intact individual."

While these investigations were limited solely to nonsystematic descriptive observations, there is no reason to question their basic validity. The key to understanding the lack of visual deficits that they describe may lie in the fact that all their experiments were conducted on various species of dogfish, most often *Scyllium*. The findings of Veselkin and Kovacevic (1973) imply that these sharks may be unique among elasmobranchs and lack the physiological basis for telencephalic visual involvement.

In view of the unexpected findings that resulted from using instrumental conditioning to analyze tectal lesions, we thought that a similar approach might prove equally revealing in reassessing the effects of telencephalic ablation.

Telencephalic Ablation and Visual Discrimination

Postoperative Learning of Novel Tasks—The initial experiment was designed along the lines of the previously described tectal study. Two groups of juvenile nurse sharks were trained postoperatively to discriminate black from white (BW) and horizontal from vertical (HV) black and white stripes. One group received bilateral suction lesions aimed at destroying most of the central telencephalic nuclei, especially the posterior portion known to receive input from the visual area of the thalamus. The other group underwent bilateral aspiration of the anterior telencephalon and thus served as an operated control group. The training and histological procedures were identical to those already described for the tectal experiment, with only one shark (NS-191) receiving any preoperative experience in the training situation.

The operated control subjects exhibited no substantial behavioral deficits other than anorexia in the first two weeks after surgery. However, none manifested any subsequent motivational deficits during training. Their learning

performance is depicted in Figure 5. Both discrimination tasks were learned within 40 training sessions or fewer, with the stripes discrimination requiring slightly more trials for each shark. Although subject NS-185 did not reach criterion on BW until session 28, it performed significantly above chance by session 15, according to a "runs" analysis of successive correct responses (p < 0.01; Runnels et al. 1968). Histological analysis revealed that the rostral third of the telencephalon, including portions of the lateral olfactory area, was severely damaged in all three subjects.
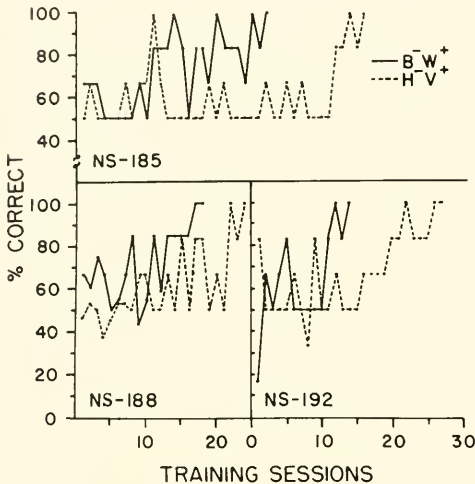
Figure 5 Postoperative learning curves for nurse sharks with bilateral anterior telencephalic control lesions. Performance is plotted as a function of the number of trials after starting each task, BW preceding HV in each case.

The sharks with more posterior telencephalic lesions exhibited varying degrees of visual dysfunction, depending on the extent of damage done to the central telencephalic nuclei. One of the sharks had a lesion that extended only slightly more caudally than the control lesions and did not disrupt the central nucleus (except for its rostral pole) or its pathways. As shown in Figure 6, the performance of this shark (NS-190) did not differ much from that of the controls. Subject NS-189 performed more poorly on the BW task, developing position habits that had to be corrected during training. Also, in spite of some obvious positive transfer, it was unable to reach criterion on the HV task in the limited time available. The lesion in this shark was located farther caudally than that in NS-190 and entirely destroyed the left central nucleus and portions of the right one, transecting both the afferent thalamo-telencephalic tract and the efferent tractus Pallii on the right side.

The other two subjects in this group were unable to learn either discrimination task, despite the lack of any motivational impairment. The results of their training are shown in Figure 7. The progressive decrease in median correct response latency indicates that NS-186 successfully learned the general response requirements of the training procedure and could correct errors quickly; however, its performance on the BW discrimination task continued to vary about the chance level throughout the 522 trials. Near the end
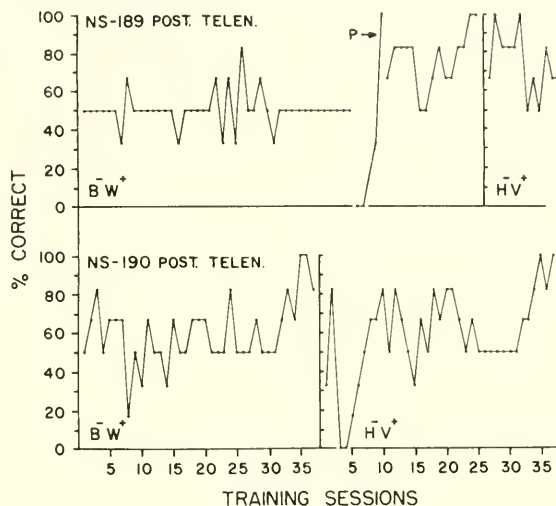
Figure 6   Postoperative learning curves on the BW and HV tasks for two sharks with bilateral lesions in the posterior telencephalon. "P" indicates attempt to correct position habit by placing correct target on nonpreferred side for all daily trials.

of BW training the shark developed a position habit that persisted into HV training despite attempts to correct it. The visual nature of this shark's postoperative learning deficit is confirmed by its subsequent performance on a nonvisual, position task in which the correct target of the HV pair was always located on the left, or nonpreferred, side. The shark reached criterion quickly, after 54 trials. It is clear that the shark did not depend on visual target cues to learn this later task, because it continued to choose the left-hand target when the positions of the horizontally and vertically striped targets were reversed after criterion was achieved.

NS-191 required more training than NS-186 before its median daily response latency decreased to a stable, low level, even though it had received 14 days of pretraining before undergoing surgery. The improvement in response latency coincided with the appearance of vicarious trial-and-error behavior and sudden swerving from one side of the approach alley to the other before choosing a target. Upon the failure of any stable performance to develop on the BW task, the shark was switched to the more difficult HV task. Here training was finally halted after the subject became uncooperative, frequently circling just before the targets and refusing to swim through parts of the conditioning apparatus.

The histological findings for these two sharks are reconstructed in Figure 8. They both received substantial damage to the portions of the central telencephalic nucleus receiving input from the thalamic visual areas. Despite their poor performances on the visual discrimination tasks, subjects NS-186 and NS-191, like the other sharks, were able to detect the presence of light,
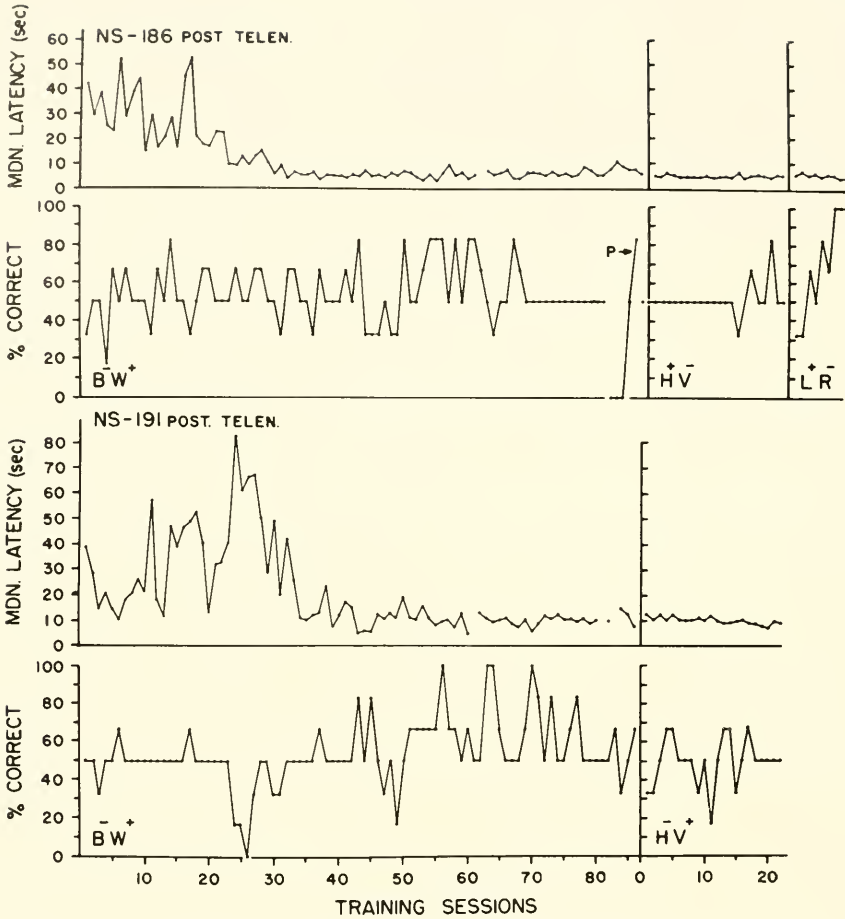
Figure 7 Postoperative learning curves on the BW and HV tasks for two sharks with bilateral posterior telencephalic lesions that subsequently damaged areas of the central telencephalic nucleus receiving visual input. Upper function in each pair indicates median daily response latency, from leaving start pen to making correct response.

as indicated by their quickly turning away from a light source suddenly switched on during darkness. In addition, they all exhibited normal pupillary responses to changes in ambient light intensity.

These findings offer the first behavioral evidence for telencephalic visual mechanisms in sharks and differ significantly from the results of similar studies with teleost fish, in which no postoperative visual deficits have been found (Bernstein 1962, Iwai et al. 1970, Nolte 1932, Savage 1968a, Savage and Swingland 1969). The reported decreases in spontaneous activity and increases in response latency and variability in teleosts (Aronson and Herberman 1960, Dewsbury and Bernstein 1969, Kaplan and Aronson 1967, Overmier and Curnow 1969) were not observed in the sharks with posterior telencephalic lesions and therefore cannot be used to explain their poor

NS-186
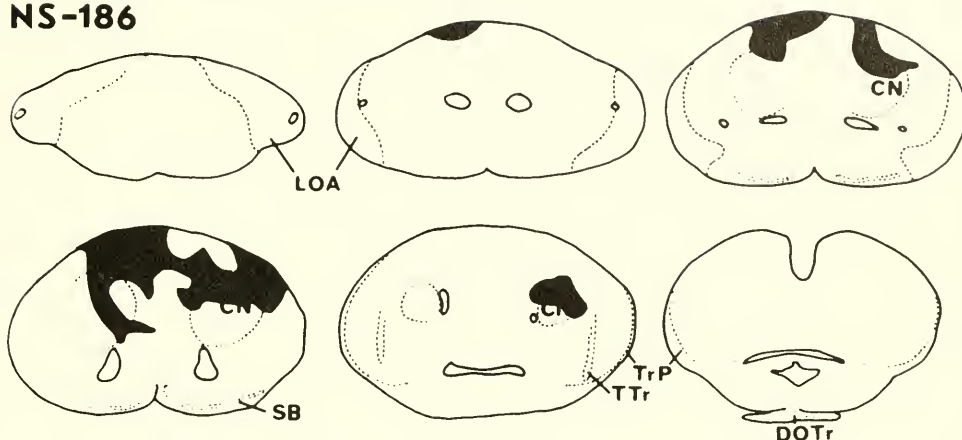
NS-191
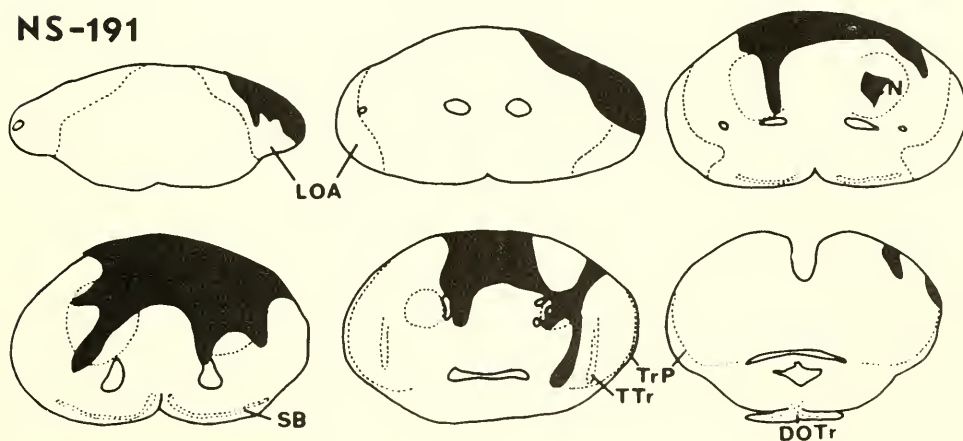
Figure 8 Transverse reconstruction of lesions for subjects NS-186 and NS-191. Sections are ordered rostrocaudally, 2 mm apart, with black areas indicating missing or necrotic tissue and ventricles outlined by solid black lines. Abbreviations are CN, central telencephalic nucleus; DOTr, decussation of the optic tract; LOA, lateral olfactory area; TrP, tractus pallii; TTr, thalamotelencephalic tract; SB, area superficialis basalis.

performances on the BW and HV discrimination tasks. NS-186's rapid learning of the position task is especially inconsistent with this type of explanation and also argues against any disorder of short-term memory or reinforcement mechanisms, as reported in teleosts with telencephalic lesions (Flood and Overmier 1971, Savage 1968a, 1968b, 1969a, 1969b).

Effects of Telencephalic Lesions on Learned Visual Discrimination Habits—The surgically induced impairments in shark visual discrimination

learning could have resulted from any of a number of types of visual dys-
function. The most obvious would be a purely sensory loss causing an
inability to distinguish between the targets. The best way to verify this
would be to examine the effects of such lesions on classically conditioned
discriminations, thus experimentally removing any deficits due to abnormal
orientation or voluntary motor mechanisms.

Because such a study was not possible at the time, a second approach was
chosen, aimed at determining whether the previously observed losses were
caused by the sharks' inability to learn to use visual cues to guide their swim-
ming movements (Graeber et al., in preparation). The extent of post-
operative training was minimized by training the subjects to criterion on the
visual discrimination tasks before surgery. It is possible that central
telencephalic lesions affect only visual learning capacity; if so, the sharks
would retain the learned habits after surgery. The alternative outcome is that
they would have to totally relearn the discriminations, this would indicate
that such neural damage interferes with the ability to discriminate (i.e., a
sensory loss) or to use visual cues to guide behavior (i.e., a visuomotor loss).

In analyzing the results the sharks were grouped according to the extent
of damage to the central telencephalic nuclei. The performance of the sub-
ject with the most extensive damage is shown graphically in Figure 9. Before
undergoing surgery, NS-530 learned both tasks rapidly and retained them al-
most perfectly after 28 days of rest. It began eating well two days after
surgery and then resumed training.

During the first three sessions the shark was very difficult to train, startled
easily, and refused to swim through the gates. When not being trained it
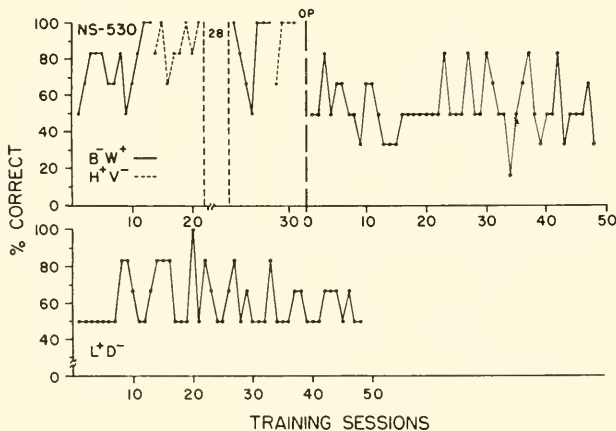
Figure 9   Performance of NS-530 on visual discrimina-
tion tasks before and after surgery. The 28-day retention
period is indicated by the area between the pair of
vertical dashed lines, and the point at which surgery
was performed is indicated by the dashed line marked
OP. The lower graph shows performance on light-dark
(LD) task after postoperative BW training.

typically remained on the bottom of the pool unless prodded, to which it responded by suddenly swimming away erratically, often colliding with the walls and other sharks. After two more sessions, this behavior became less pronounced, but the shark still refused to approach the stimulus targets directly and hesitated while swimming back to the start pen after each trial. It usually chose a side as soon as it exited the start pen and then swam up the alley while maintaining contact with one wall. The choice of sides appeared to vary randomly and thus did not resemble the position habits seen in other sharks. The animal never performed above chance on BW, even though it continued to eat and swim well.

Its subsequent performance on a nighttime light-dark discrimination task, shown in the lower panel of Figure 9, suggests that the shark could detect the presence of light; responding often exceeded chance but never dropped below it. However, the shark never reached criterion and was finally sacrificed after 48 sessions. Post-mortem examination revealed a large lesion that removed about 95% of the central telencephalic areas that receive thalamic fibers (Figure 10).
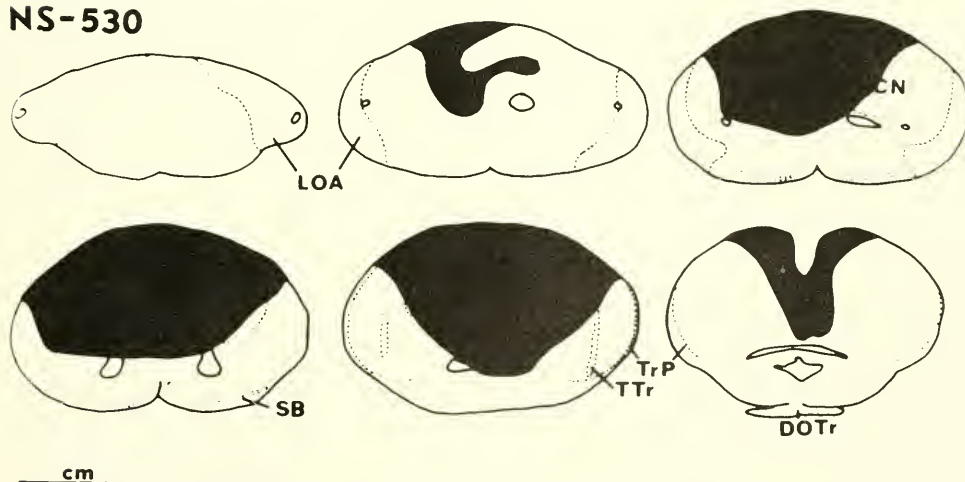
Figure 10   Transverse reconstruction of lesion for subject NS-530. See Figure 8 for abbreviations.

Figure 11 presents behavioral results for three more sharks with lesions involving between 10% and 50% of the central telencephalic nuclei and inflicting no damage to their connecting pathways. While only NS-501 exhibited any significant postoperative retention of either task, these subjects were less severely affected than NS-530 but still had to be retrained. There was some saving on BW but none, or very little, on HV. Three other nurse sharks, in which 10% or less of the central nuclei was damaged, showed almost perfect retention of the preoperatively acquired discrimination habits (Figure 12).
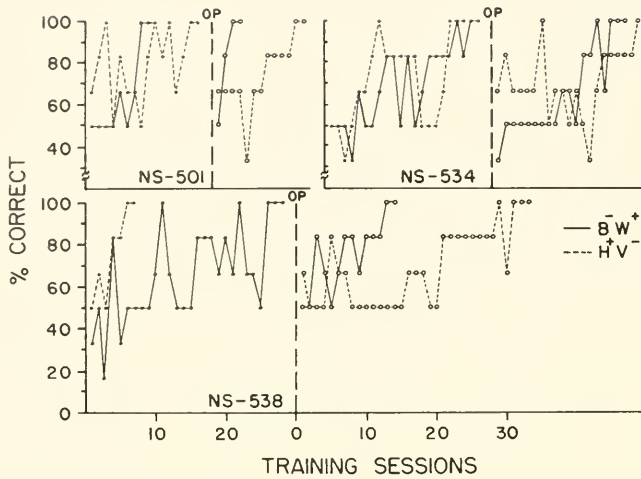
Figure 11 Performance of three sharks on the BW and HV discrimination tasks before and after surgery (OP) that damaged 10% to 50% of the central telencephalic nuclei.
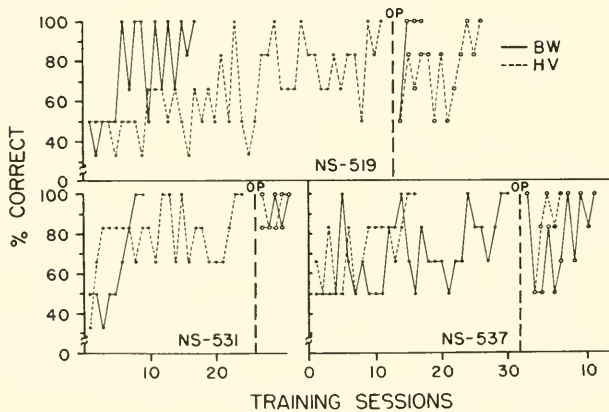
Figure 12 Performance of three sharks on the BW and HV discrimination tasks before and after surgery (OP) that damaged less than 10% of the central telencephalic nuclei.

The results of this second experiment indicate that the discrimination deficits seen so far are not due to disruption of visual learning mechanisms alone, but can be attributed instead to some type of sensory loss or sensorimotor loss that makes it difficult for them to orient to, or localize, a stimulus in visual space. It is still difficult to decide exactly which type of loss is responsible. The animals with severe central telencephalic damage can detect light and are therefore not totally blind. This conclusion agrees with Baru's finding that sharks with more complete telencephalic ablations are

able to learn and retain classically conditioned reflexes to light onset (Karamian 1956). The misdirected approach behavior seen in subjects with substantial damage to the central telencephalic nuclei argues for the presence of an orientation deficit. Moreover, it appears to be specific to localizing visual stimuli since preliminary results indicate that such sharks can still localize low-frequency, pulsed sound sources (Thomas and Jane, personal communication).

While our current knowledge does not permit a final conclusion about the nature of the observed behavioral deficit, there is no doubt that the behavioral evidence supports the corresponding anatomical and electrophysiological data in affirming the presence of telencephalic mechanisms in the shark central visual system.

An Integrated View of Central Visual Components

The classic belief in tectally dominated vision in sharks at least had the virtue of simplicity. We must now try to understand the functional interrelationships that govern the operation of several central visual system components. While this chapter has dwelt on two of the most prominent, the tectum and the central telencephalic nuclei, other visual regions need to be addressed. Most of these are in the thalamus, which has been shown (Ebbesson and Ramsey 1968, Graeber and Ebbesson 1972b) to have three distinct areas receiving retinal terminations: the dorsomedial optic nucleus or pretectal area, the dorsolateral optic complex, or lateral geniculate nucleus (Houser 1901), and the ventrolateral optic nucleus. These three areas are also connected anatomically with the tectum and telencephalon (Ebbesson 1972a, 1972b, Schroeder and Ebbesson 1974), but at the present time it is not known for certain which of the three contribute to the visual input reaching the latter. The presence of a posterior accessory optic tract, similar to that seen in mammals, has also been reported in the thalamus, but only in tiger sharks (Ebbesson and Ramsey 1968). Finally, in all species examined so far, there are a small number of retinal fibers that leave the optic tract just after it decussates and enter the contralateral hypothalamus, where they may be involved in the control of endocrine function and circadian rhythms.

Some work that begins to separate the functional interrelationships between these different central visual areas has already been described. Specifically, Veselkin and Kovacevic (1973) found that telencephalically evoked responses to optic nerve stimulation were retained in rays after complete destruction of the midbrain optic tectum. It appears therefore that the retinal input to the dorsolateral thalamus, rather than the overlapping tectal input, is being relayed to the contralateral central telencephalic nucleus. This finding may explain why we found no serious impairment in visual discriminative learning from tectal lesions. Both the physiological and behavioral results suggest that there may be two functionally different subsystems in the overall elasmobranch central visual system: a midbrain retinotectal system and a forebrain retino-thalamo-telencephalic system. Furthermore, these subsystems may be partly redundant, as indicated by the extent of

anatomical overlap seen in the retinal and tectal terminations in the dorsola-teral optic complex of the thalamus.

As I pointed out earlier, it is difficult to draw firm conclusions about the exact nature of the behavioral deficits caused by telencephalic lesions with-out further data from experiments that carefully separate the response re-quirements of the postoperative test situation. It is equally premature to discuss the deleterious effects that tectal ablation must have on some aspects of visual functioning. Nevertheless, in that the deficits so far observed with central visual lesions involve visually guided behavior, there is a temptation to speculate about the functional differences between the two proposed cen-tral visual subsystems.

One hypothesis is raised by studies that show that visually elicited behaviors can be experimentally distinguished from visually guided behaviors in kittens (Hein and Held 1967, Hein and Diamond 1971). Eventually it may be shown that the shark tectum is involved primarily in the control of visually elicited behaviors, while forebrain visual mechanisms are concerned with more voluntary, visually guided behaviors. Contrary to Schneider's (1969) results with hamsters, the successful discrimination learning of the tectally ablated subjects indicates that this structure is not seriously involved in the shark's ability to voluntarily orient toward an object. Their performance further demonstrates that the shark tectum is not responsible for learning to identify visual stimuli. Where no direct data are available on the effects of tectal lesions on visually elicited behaviors in sharks, substantial evidence in the fish literature confirms that the tectum governs such behavior in teleosts (e.g., Akert 1949). Hopefully, increased testing of unlearned responses and the continued use of conditioning techniques will help us further unravel the functional aspects of the shark's central visual mechanisms.

SUMMARY

Recent advances in the sensory biology and neuroanatomy of elasmobranchs have enhanced the chances for success in studying the brain—behavior relationship in sharks. The advantages of such an analysis are discussed in relation to past efforts to understand shark behavior and specifically in terms of learning more about shark central visual mechanisms. The morphology of the shark's central visual system is reviewed in light of new findings compared with the still prevalent traditional views of comparative neuroanatomy. Following a discussion of various methods to assess visual function in sharks, behavioral results are presented which indicate that the optic tectum does not exert exclusive control over visually guided behavior in sharks. The participation of the telencephalon in central visual processing is supported by a combination of anatomical, electrophysiological, and behavioral data. Nurse sharks with experimental lesions of the visual portion of the central telencephalic nucleus have difficulty learning, or are unable to learn, simple visual discrimination tasks. The fact that earlier workers reported no visual deficits following telencephalic ablations may have been due to their exclusive use of dogfish, the thalamotelencephalic pathways of which have no visual function. The exact nature of any functional differences between the midbrain and forebrain components of the shark central visual system remains a matter for conjecture.

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III CHEMICAL SENSES

ELECTROPHYSIOLOGICAL STUDIES OF CHEMORECEPTION

IN ELASMOBRANCHS

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Dr. E. S. Hodgson filming shark behavior from elevated observation platform (upper photo); Dr. R. F. Mathewson prepares to attach blinker light to lemon shark (lower photo).

Receptor cells sensitive to chemicals are among the most important compo-
nents of elasmobranch sensory systems. Such cells are *chemoreceptors*. By
convention, chemoreceptors are divided into *gustatory* (taste) receptors and
olfactory (smell) receptors. A third "common" or "general" chemical sense
has been postulated (Parker 1922, Tester 1963), but it is involved in re-
actions to relatively high concentrations of chemicals (often so-called irri-
tating compounds) and it appears to lack the specificity and specialized
end organs of olfaction and gustation. This review will be limited to the
olfactory and gustatory receptors, with special emphasis on the modern
techniques of electrophysiology that are being used increasingly for their
study.

HISTORICAL DEVELOPMENT OF STUDIES
ON CHEMORECEPTION

Scientific understanding of the chemical senses of elasmobranchs, and most
animal sense organs, has developed through three general stages. The first
is characterized by observations of the animals in their natural surroundings.
The information gleaned from such observations is at first anecdotal but may
become organized and codified into understanding that can be valuable in
pointing the ways to appropriate experimental analyses. Examples of such
insights concerning the sensory biology of sharks, incorporated into the
traditions of seagoing Pacific cultures, are presented elsewhere in this volume
(Hodgson 1978).

A second stage of understanding is reached when the *structures* of the
sense organs that mediate particular behavioral patterns are determined.
Anatomical investigations of the olfactory and gustatory sense organs of
elasmobranchs dominated the studies in this field from the 1860s until well
into the present century, and have been extensively reviewed by Parker
(1922), Tester (1963), and Kleerekoper (1978).

The third stage is physiological analysis. This level of understanding
relies heavily on electrophysiological techniques.

It is clear from any survey of progress in this field that these stages of
historical development are not mutually exclusive. For example, some types
of anatomical studies, particularly those at the level of cellular ultrastruc-
ture, are extremely important today. Moreover, the electrical techniques
of neurophysiology have not preempted the approaches essential for modern
physiologists working in this area. There has been a retreat from an initial
assumption that nerve recordings from chemosensory organs would corre-
late exactly with behavioral reactions of intact animals stimulated by chemi-
cals. This revision of viewpoint is not confined to studies of elasmobranchs,
but applies to other forms studied by electrophysiological methods as well
(Hodgson 1965). Thus, it is clear that new physiological studies of the
chemical senses require a return to (and more precise experimental design of)
continuing behavioral studies on whole animals.

In the future there may be a fourth stage of analysis, concentrating on
the "coding" of afferent impulses from chemoreceptors and on their inte-

gration with other activity in the central nervous system, which undoubtedly influences patterns of efferent potentials controlling swimming, orientation, and other behavior. However, analysis of these neural integrative mechanisms is still in its infancy.

The beginnings of electrophysiological approaches to the chemical senses of aquatic vertebrates are noted in G. H. Parker's (1922) classic book, *Smell, Taste, and Allied Senses in the Vertebrates*. Parker reported that a weak electrical stimulus seemed ". . . in every way to duplicate the stimulus normal for the organ of taste" in certain fishes. In fact, if the currents led into aquaria through water-filled glass tubes were reduced to less than a microampere, catfish (*Ictalurus*) would approach the open ends of the tubes and ". . . nibble at the current as though it were a bait." Although at that time Parker was actively investigating the responses to chemicals by sharks, he apparently did not extend his observations on electrical stimulation to any of the elasmobranchs.

During the 1930s, the first neurophysiological recordings were made from chemosensory systems of fishes. Nerve discharges from gustatory fibers of the facial nerve, innervating the barbels of catfish, were recorded by Hoagland (1933). Shortly thereafter, Adrian and Ludwig (1938) achieved the first recordings from the olfactory nerve of the same species. These early studies hinted at some properties of the chemosensory systems, and experimental studies of them, that are still noteworthy. The effective chemical stimuli were found to initiate impulses (action potentials) of lower amplitude than those from mechanoreceptors—an observation frequently confirmed in subsequent studies, and one that is obviously important for experimental design and the interpretation of results.

In this period, when investigators sought to apply the new electrophysiological techniques to a wide variety of aquatic animals, it seems significant that the first notable successes were achieved with experiments on freshwater rather than marine species. Undoubtedly the lower conductivity of freshwater, which lessened the problems of short circuiting between electrodes, influenced the ease with which results could be obtained using the recording techniques then available. It is noteworthy also that none of the early techniques recorded electrical events in actual chemoreceptor cells, but only in sensory nerve fibers supplying the receptor cells.

The 1950s and 1960s were a period of rapid advances in neurophysiological analysis of chemosensory systems. Hodgson, Lettvin, and Roeder (1955) used the special anatomical advantages of insects to obtain the first records of electrical activity in primary chemoreceptor cells. Rapid strides were made in experiments on the chemical senses of insects and mammals, summarized in the international symposia on olfaction and taste, a series of conferences that still continues (e.g., Zotterman 1963, Hayashi 1967, Schneider 1972).

Despite new findings from studies on insects and mammals, which had important implications for concepts of sensory physiology as a whole (Beidler 1970, Hodgson 1965), the experimentation on groups other than mammals and insects was sparse; among aquatic animals it was limited to

teleost fishes (Bardach et al. 1967). It was not until the mid-1960s that electrophysiological techniques were applied to studies of the chemical senses of elasmobranchs, when Gilbert, Hodgson, and Mathewson (1964) recorded changes in electroencephalograms following chemical stimulation of sharks.

BASIC THEORY

In considering the prospects and limitations of electrophysiological analyses of the chemical senses of elasmobranchs, it is appropriate to consider first the types of electrical potentials that may be recorded by various methods. An evaluation of these possibilities will also help explain why electrical recording methods were applied relatively late to chemoreceptors.

The small sizes and relatively inaccessible locations of most primary chemoreceptor cells have posed serious problems for practical experimentalists. The small size of the cells means that the electrical potentials they generate are also small, often below the amplitude of the electronic noise levels of available amplifying systems. Penetrating the cells by microelectrodes, an often useful method of improving the signal-to-noise ratio in neurological studies, is typically ruled out by either small size or inaccessibility of the primary chemoreceptor cells. When those difficulties can be overcome, however, the electrical phenomena associated with chemosensory functions have proved less diverse than the anatomical details of the sense organs and their associated structures. For the latter reason, it seems justified to draw on comparative data to provide useful theory about those points of elasmobranch chemoreception for which data on elasmobranchs remain inadequate. Data from teleost fishes, other vertebrates, and even insects can be helpful in this regard and will be referred to, as appropriate, throughout this review.

Generator Potentials

The electrical symptoms closest to initial stimulation of a receptor cell are the *generator potentials*. They are graded, sustained electrical potentials, arising at primary sites of stimulation and preceding the afferent impulses (Figure 1A).

Granit (1955) developed much of the early theory of generator potentials and has provided important reviews of their significance. Because of their closeness to initial transduction processes of stimulation, physiologists concerned with basic mechanisms of stimulation prefer to record generator potentials wherever possible. Although this has been done in certain experimental preparations of insect and mammalian chemoreceptors, it has not yet been achieved with elasmobranchs. When this level of analysis is reached with elasmobranchs, it will be necessary to bear in mind an assortment of constraints on the definition of true generator potentials. These are beyond the scope of the present discussion but have been reviewed by Hodgson (1965).
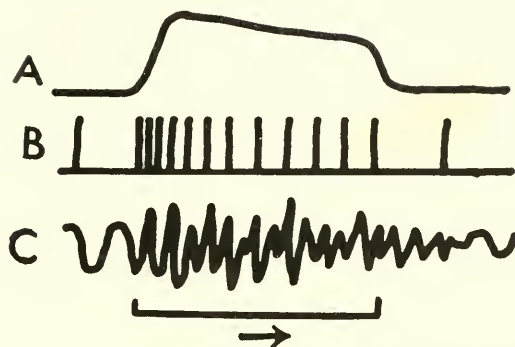
Figure 1 Types of electrical potentials recorded by various techniques: (A) generator potential, (B) action potentials, (C) electroencephalogram. Lower line indicates duration of applied stimulation, with the time sequence reading from left to right. Note that some "spontaneous" impulses and EEG potentials preceed and follow the period of stimulation in these examples.

Electro-Olfactograms (EOG) and Related Potentials

In the 1930s it was discovered that when odors were applied to olfactory epithelia of various mammals, a slow electronegative potential appeared. Ottoson, during extensive studies on this phenomenon in frog olfactory epithelia, proposed the name "electro-olfactogram" (EOG), and a similar potential in olfactory organs (antennae) of insects led to the term "electro-antennogram" (Ottoson 1963, Schneider 1963).

The shapes and durations of these slow potentials from the olfactory organs differ according to the type of stimulus used; consequently, they can be convenient tools for analysis. EOGs or EAGs obviously resemble generator potentials. Because of their complexities and their origins from the summed potentials of many cells, however, they do not meet the more rigorous criteria by which generator potentials are characterized. Although attempts have been made to do so (Hodgson and Mathewson, unpublished), these potentials have yet to be recorded in any consistent fashion from chemosensory organs of elasmobranchs.

Action Potentials (Impulses)

Excitation of chemoreceptors is eventually communicated to the brain as a change in afferent nerve impulses, action potentials, spike potentials, or "impulses" (Figure 1B). Despite the implications of the name, these potentials may not simply appear during chemical stimulation and disappear when stimulation ceases. In many cases a "spontaneous" afferent flow of action potentials is *decreased* during chemical stimulation. Consequently, the investigator must be prepared to decode *any* type of change in afferent action

potentials as a possible signal of significance to the central nervous system (CNS) (Hodgson 1965). Similar kinds of spontaneous activity occur in the ampullary electroreceptors of elasmobranchs, and are discussed elsewhere in this volume by Bennett and Clusin (1978).

Experimental preparations of single chemosensory afferent nerve fibers, or "few-fiber" preparations (which permit recognition of impulses from different cells on the basis of their different impulse voltages) have been achieved in a number of fishes (e.g., Bardach et al. 1967). They have been the object of investigation in several elasmobranchs. Tester (1975) reported that action potentials had been recorded from the olfactory tracts of hammerhead sharks (*Sphyrna*) during chemical stimulation. However, a later reevaluation of the records led to the conclusion that the impulse patterns actually had no correlations with amounts or types of chemical stimulation (Tester, personal communication). Attempts to obtain recordings from few-fiber preparations of the olfactory tract of various sharks and rays have also failed to yield reproducible results (Hodgson and Mathewson, unpublished).

Olfactory Bulb and CNS Recordings

Because the central neuropil is a complex mixture of very fine nerve processes, the electrophysiological methods usually applied to single cell units can rarely be used in the central nervous system (CNS). Multi-unit recordings, used with CNS preparations, include recordings of evoked potentials, synchronized responses, and rhythmic electroencephalogram (EEG) patterns. It was the latter method that first demonstrated electrical correlates in the elasmobranch nervous system during stimulation by chemicals (Gilbert, Hodgson, and Mathewson 1964). An example of such an EEG pattern is diagramed in Figure 1C. Eventually, this technique was refined so that chronically implanted electrodes could record EEGs in swimming sharks, which made possible the correlation of brain potentials with behavioral responses during controlled stimulation by chemicals (Hodgson, Mathewson, and Gilbert 1967). In addition to the changes in rhythmic potentials in the forebrain during chemical stimulation, there are changes in the medulla associated with gill movements during chemical stimulation. In general, the EEG responses of sharks and rays appear similar to those of teleost fishes. The larger sizes of some of the shark brains studied may be an advantage over some of the teleost preparations used in experiments, and this may explain some earlier reports of failures to detect forebrain responses in teleosts during chemical stimulation e.g. Adrian and Ludwig 1938).

Evoked potentials, following electrical stimulation of the olfactory mucosa, have been recorded from various parts of the olfactory bulb and forebrain in the shark *Scyliorhinus* and in *Torpedo* (Bruckmoser and Dieringer 1973). The evoked potential studies have been focused mainly on the structure and circuitry of the olfactory bulbs and the secondary olfactory areas of the forebrain hemispheres. Indications are that the

olfactory bulbs and secondary projection areas have a quite uniform and conservative structure and circuitry in all vertebrate groups. Consequently, it is reasonable to use comparative data when observations are lacking on a particular species. This does not imply, of course, that the effective chemical stimuli are the same or that the ultimate behavioral responses are similar throughout the elasmobranchs and other vertebrates.

Limitations of Techniques

Ideally, experimentation on the chemical senses of any species would span the whole range of phenomena, from primary receptor cell recordings to the behavior of freely moving animals. Practically, experimenters always have to settle for only part of this range. With elasmobranchs, the closest it has been possible to come to recording of initial excitatory states are EEG-type records from olfactory bulbs. Such records have notable limitations: (1) they are not from the actual chemosensory cells, but from second-order neurons, and (2) the EEG cannot discriminate between stimuli that evoke very different types of behavioral responses. Therefore, it is absolutely necessary to return to behavioral studies, or to carry out the electrophysiological and behavioral studies in parallel, if the EEGs are to be interpreted in terms of the positive or negative effects of chemical stimuli.

A striking illustration of this need was recently reported from studies on salmon (*Oncorhynchus*). A chemical stimulus (l-serine) washed off mammalian skin has been shown to be strongly aversive to sockeye salmon (Idler, Fagerlund, and Mayoh 1956); it elicits EEG responses as large as or larger than the home stream waters, to which the salmon orient very strongly (Bodznick 1975). State of sexual maturity and recent experience of the fish have also been suggested as influences on EEG size and changes (Oshima et al. 1973, Bodznick 1975). It has become quite clear that EEG size is affected by some factors other than odor qualities of stimulus waters, leading some investigators to claim that there is ". . . an inherent lack of precision associated with evoked bulbar recordings" (Dizon, Horrall, and Hasler 1973). Some of this variability may be accounted for by the known integration of olfactory EEGs with visual, auditory, and gustatory senses (Harada and Takagi 1961), which further emphasizes the need for carefully controlled behavioral studies to accompany electrophysiological experiments involving any kind of EEG recordings.

In our own studies, detailed below, this necessity for combining electrophysiological and behavioral analyses has been applied to most of the work with elasmobranchs. Few chemical stimuli, among the wide variety tested, are without some effects on the sharks tested, at least on initial exposure. The effects may show up in EEG recordings, or they may consist of behavioral changes that indicate "awareness" of the stimulus by the shark. The presence of EEG changes alone is not predictive of a shark's behavior. The difficulties of a single approach can be largely overcome by combining EEG studies with behavioral studies.

ELECTROPHYSIOLOGICAL CORRELATES WITH
CHEMICAL STIMULATION IN ELASMOBRANCHS

Laboratory Experiments on Curarized Sharks

Not surprisingly, the first successful electrophysiological recordings from elasmobranch chemosensory systems were obtained from laboratory preparations of sharks immobilized with d-tubocurarine, using crude extracts of biological materials known to evoke feeding behavior (Gilbert, Hodgson, and Mathewson 1964). Three species were studied: bonnet sharks (*Sphyrna tiburo*), nurse sharks (*Ginglymostoma cirratum*), and lemon sharks (*Negaprion brevirostris*). The latter two species were the most extensively studied with these techniques in subsequent experiments.

Figure 2 shows the locations at which recordings were made from the brain of the lemon shark; recording sites on the brains of the other species were similar, allowing for more species differences in proportions of different brain lobes. Full technical details have been reported (Gilbert et al. 1964). The sites yielding the largest amplitudes of EEG changes, correlated with chemical stimulation from perfusion of the olfactory sac on the homo-
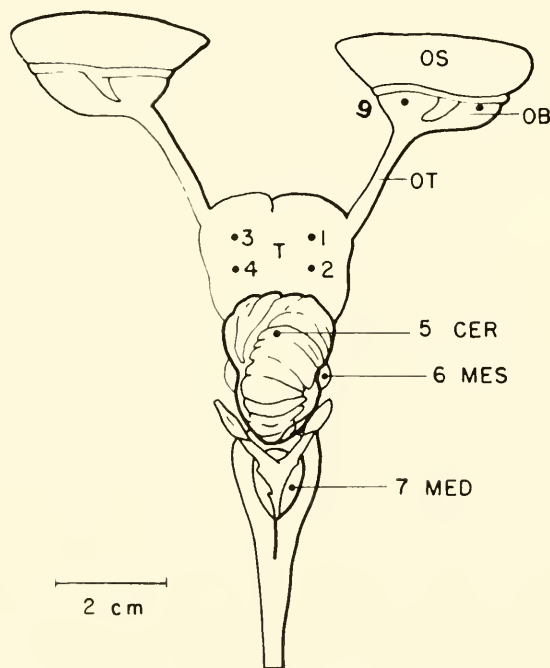
Figure 2 Brain of lemon shark, *Negaprion brevirostris*. Numbers indicate points for electrophysiological recordings. OS—olfactory sac; OB—olfactory bulb, OT—olfactory tract, T—telencephalon, CER—cerebellum, MES—mesencephalon, MED—medulla. Numbers indicate positions of electrodes in various experiments; for details, see text.

lateral side, were located closest to the point of connection between the olfactory tract and the telencephalon, either on the forebrain surface or at a depth equivalent to the distance to the center of the olfactory tract. The most effective stimuli tested in the initial experiments were extracts of tuna meat, although extracts of crabs (normal food for bonnet sharks) and "amine F" (an olfactory attractant for lampreys, fishes, and sharks, since identified as iso-leucine-methylester (Kleerekoper 1978)) were nearly as great. The exact polarities and frequencies of EEG changes, following chemical stimulation, depended on the species, recording sites, electrodes, time relationships to initial stimulation, and several other factors in addition to the chemical stimulus. Typical results are shown in Figure 3, which illustrates both the delay in onset after stimulation (latency) and the persistence, at declining levels (adaptation) of the responses after initial maxima.

Since no previous studies have been made of EEGs in this group of animals, it was necessary to characterize the relevant patterns of electrical activity in the forebrain and elsewhere. Spontaneous fluctuations of potential in the telencephalon, with a dominant frequency of 4 to 9 Hz, were commonly recorded from the brain surface; both amplitudes and frequencies of these potentials increased during chemical stimulation (Figure 3B). The 4- to 9-Hz rhythm is similar to one recorded from the telencephalon of the goldfish (Schadé and Weiler 1959); however, a second, more rapid rhythm reported in the goldfish forebrain was not observed in the sharks. Spindling was rare, and was usually associated with the pressure of surface electrodes or movement of depth electrodes, as has been noted in EEG studies on codfish (Enger 1957).

Among the significant EEG patterns found in other areas of the shark brain is a medullar pattern consisting of relatively large-amplitude potentials (150 to 200 μV), correlated with gill movements. These potentials appear to be associated with neural triggering of respiratory reflexes. Although the medullar potentials were sporadic in the restrained curarized sharks while they were maintained in the laboratory with seawater perfusion through the gills, these particular EEG patterns were subsequently found to be quite useful in recording responses to chemicals by unrestrained swimming sharks (see below).

To summarize, the initial studies of EEGs established several characteristics of the shark brain responses during adequate chemical stimulation of the olfactory sac: (1) the typical response was an increase in amplitude and frequency of whatever spontaneous potentials could be recorded at a particular site, (2) such responses were given to extracts of normal food materials, as well as to several pure chemicals (amino acids and amines) present in the normal food, and (3) latency and adaptation of the responses evoked by sustained administrations of stimuli at constant levels were always present. From the restrained and curarized sharks, nothing could be concluded about the behavior that might normally have been associated with these EEG responses. Consequently, the experimental approach was modified so that EEGs could be recorded from unrestrained free-swimming sharks exposed to predetermined concentrations of pure chemical stimuli.

Figure 3 Electroencephalographic records from forebrains of sharks. Broad horizontal bars indicate periods of chemical stimulation. The time scale, as indicated, is the same in all records. Vertical calibrations 50 μV in all records. (A) forebrain surface, bonnet shark, during perfusion of olfactory sac with seawater; (B, C) same, during and following chemical stimulation; (D) forebrain surface, nurse shark, with pure chemical stimulus; (E to H—depth recordings from the forebrain of lemon shark, stimuli as indicated.

Combined EEG/Behavioral Studies on Free-Swimming Sharks

The apparatus used for studies on free-swimming sharks is shown in Figure 4. A 1150-gal. circular flow system was driven by propellers so that the seawater moved through a hydrodynamic tunnel at a uniform rate on all levels, without appreciable turbulence. Chemicals introduced near the propellers circulated in precisely known dilutions and time intervals, passing through the glass observation chamber.

After a brief period of acclimatization when first placed in the observation chamber, sharks took up their regular unstimulated activity patterns within the chamber. Lemon sharks swam continuously but slowly. Nurse sharks settled on the bottom of the observation chamber, snouts directed upstream; they rarely swam again until stimulated by a chemical test solution in the water current. All sharks had electrodes implanted in several locations, always including either the olfactory bulb or the anterior lateral area of the telencephalon, as described in the previous section. Wires from the implanted electrodes led through a swivel, adjusted to allow complete freedom of motion, to the EEG recording apparatus.

Figure 4   Hydrodynamic tunnel for testing responses of free-swimming sharks to known concentrations of pure chemical stimuli. Top of apparatus removed, showing channel for nonturbulent flow of seawater. Glass observation tank on right, near observer.

Motion picture films were made of the behavior of the experimental animals, simultaneously with the EEG recordings before, during, and after chemical stimulation. This made it possible to categorize behavioral responses as positive (orienting upstream, toward source of the stimulant) or negative (orienting downstream, away from source of the stimulant), and to separate and compare the evoked EEG potentials from behavioral effects. It is useful to examine one example from the experimental records in further detail, to appreciate how these EEG and behavioral responses were coordinated.

A typical EEG example is shown in Figure 5, a record made during stimulation of a nurse shark with betaine. The upper trace indicates when the stimulus could be first detected in the center of the observation tank. The middle trace is from the shark's medulla, showing the neural activity associated with respiratory movements of the gills. The lower trace records potentials between the No. 1 and No. 2 surface recording positions on the telencephalon. The horizontal time marker represents 1 s, the vertical calibration indicates 50 μV.

During the approximately 11 s of this sample record, changes in the patterns of potentials from both forebrain and medulla start almost synchronously, before the lead edge of the bolus of stimulus even reaches the center of the observation tank. There is an increase in amplitude and frequency of cycles in the forebrain, and an extra gill beat triggered from the medulla. Motion picture records show that immediately after the extra gill beat, the gills are closed for a few seconds; it is during that period, when the coverings of the gills are smoothly pressed against the side of the body, that the shark begins to swim forward, toward the source of the chemical stimulation.

The combined EEG and photographic records give a clearly integrated picture of the beginnings of a positive reaction to a chemical stimulus. Very quickly after the afferent olfactory signal arrives in the forebrain, extra oxygen is taken in as a result of modified respiratory rhythms from the medulla. Gills are closed for an interval, and during that period of maximum body streamlining the swimming reflexes are activated, propelling the shark forward toward the source of stimulation. Within a few seconds, the normal cycle of gill beats resumes, and the shark continues swimming upstream.

In some cases, recordings from the surface of the olfactory bulb could
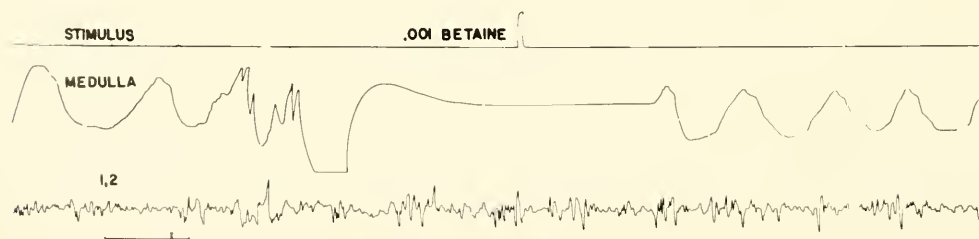
Figure 5 EEG record of reaction of free-swimming nurse shark to stimulation with betaine. See explanation in text.

be obtained under similar test conditions; this is a less successful recording technique, however, since the swimming sharks often dislodged the implanted electrodes by wiping their snouts against the walls of the observation tank during the initial acclimatization period. Figure 6 shows an instance in which pronounced rhythms were obtained from the bulb, and electrodes implanted near the tail recorded muscle potentials, providing another measure of the shark's swimming activity. It is important to note several other differences between the records shown in Figures 5 and 6. The polygraph speed in Figure 6 is slower, the horizontal calibration representing 3 s; this slower speed of the traces compresses the potentials so that they more nearly resemble (but are definitely not) afferent spike potentials. Also, the amplification of the record from the bulb has been increased, with the vertical calibration in this case being 20 μV. The record shown in Figure 6 starts when a few low-level potentials were first recorded from the olfactory bulb, at the onset of chemical stimulation. An extra gill beat and large increase in bulb activity follow, coinciding with stronger swimming movements, as indicated by the muscle potentials from the tail region.

While the overall reactions of the nurse and lemon sharks are similar, a comparison of the records in Figures 5 and 6 does reveal differences. Lemon sharks are characteristically (but not invariably) in motion at all times. Slow swimming with open mouth is a widespread method of irrigating the gills in sharks, contrasting with the pumping of the gills when nurse sharks are immobile. The amplification of the tail muscle potentials in Figure 6 has been decreased to a level where the slow minimal contractions necessary for base level swimming by the lemon shark do not produce significant fluctuations in the record. Stronger swimming, characteristic of positive responses to chemical stimuli, produces obvious deflections in the lower trace.

A second difference between the nurse and lemon sharks is seen in the medullar centers controlling gill beats. In both species, the extra beat at the onset of stimulation is seen. However, the lemon shark, while it may temporarily prolong a few gill beats (see Figure 6), does not stop its gill
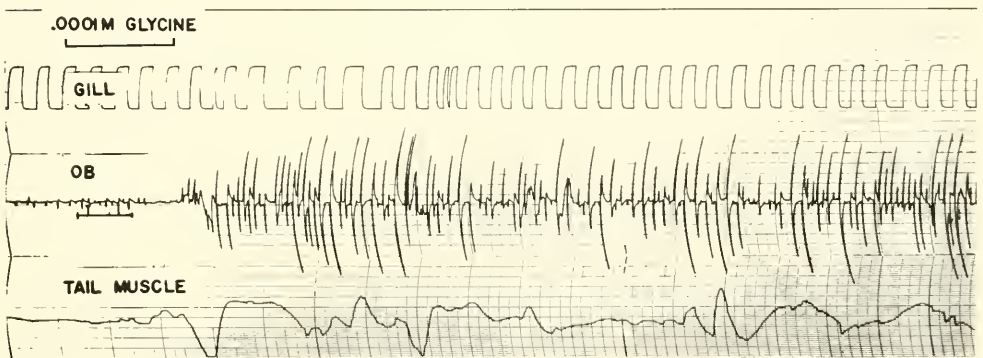
Figure 6   Reaction of a free-swimming lemon shark to stimulation with glycine. See explanation in text.

beats completely. Lemon sharks may also interpolate extra gill beats into the usual rhythm, especially when swimming very strongly, as in the Figure 6 example. These differences in the records from nurse and lemon sharks appear to be adaptations to obvious differences in the overall patterns of activity in the two species. The initial lunge of the previously immobile nurse shark may place a higher premium on streamlining, even at the cost of interrupting oxygen intake; the constantly swimming lemon shark briefly prolongs the posture of the gill covers in the closed streamlined position, but very quickly the need for oxygen to sustain its vigorous swimming takes precedence, even requiring extra gill beats to be interpolated later in some instances.

With both species, it is possible to differentiate these complete initial reactions to chemical stimuli from reactions of "awareness" only. In the latter cases, the sharks may change body posture, rearing up heads toward the stimulus source (nurse sharks) or turning toward the stimulus source (lemon sharks), with or without changes in the rhythm of gill beats, but not giving further reactions. In these cases it is clear that the animals are aware of the chemical stimuli, but do not show sustained orientation or attempts to approach or avoid the stimuli. EEG changes are rarely observed with the simple awareness responses. This undoubtedly is because the sharks can integrate and respond to afferent impulses that are too few, or too subtly different from the usual patterns of neural activity, to be recognized in the EEG traces. It is well established that swimming activity is closely associated with many areas of the CNS, including arousal, escape, feeding, and spinal centers (Demski 1977), and so there is no expectation that a strict separation of awareness responses from at least transient locomotor respones would be usual, although the snout-raising of otherwise immobile nurse sharks is one instance in which such a distinction can be made.

The problem of designating "negative," aversive, or avoidance responses is difficult when observations are confined to the glass observation tank of the hydrodynamic tunnel. In a few cases (e.g., with holothurin extracts), sharks oriented *away* from the incoming current and nosed against the restraints on the *outflow* side of the tank; these are, at least initially, negative responses. As described below, it was necessary to conduct tests in large enclosures, more closely simulating the natural open sea conditions, to determine whether orientation toward or away from these stimuli would be sustained or quickly reversed.

For those chemical stimuli that evoked strong responses, both EEG and behavioral, within the confines of the observation tank of the hydrodynamic tunnel, additional testing was done in pens measuring 40 by 80 ft (12.3 by 24.6 m). These large enclosures were situated in an area of twice daily tidal flow, in the Bimini (Bahamas) lagoon. Stimuli were introduced through Tygon tubing underwater, with release points varied to provide defined olfactory corridors, as shown in Plate I. The precise position of the olfactory corridor, and its rate of movement, depended on the tide conditions at the time of testing. Reactions of sharks were recorded by motion pictures taken from an observation platform overlooking the test pen, and also from under-

Plate 1 "Shark Chaser" being used to determine location and dilution of olfactory corridor in large observation pen. Release point is directly in front of observer. Note large lemon shark, attracted to the column of dye, turning toward the dye.

water photographic enclosures at either end of the test pen. For testing at night, the most active feeding time of nurse and lemon sharks, a 1300-lumen electron flash unit, in a floating housing, was attached to the dorsal fin of the experimental shark. By using an open-shutter photographic technique, allowing the 1/s flashes from the towed electronic flash units to mark the shark's position at 1-s intervals, it was possible to make "track records" of the orientation and swimming paths of sharks (Figures 7, 8, and 9). Further details of this method have been reported by Mathewson and Hodgson (1972).

Table 1 summarizes results of the wide variety of chemicals and extracts tested with lemon and nurse sharks in the hydrodynamic tunnel and the large observation pens. No significant differences were found in the kinds of chemicals stimulating the two test species. Significant changes in EEG patterns from forebrain or olfactory bulb are indicated by a "+" in Table 1, with unusually strong EEG effects indicated by "++." Orientation to the chemical stimulus, followed by at least brief searching or approach behavior, is indicated by a "+" in the behavioral response column. Persistence of orientation behavior beyond 10 s, or a display of "feeding frenzy" behavior
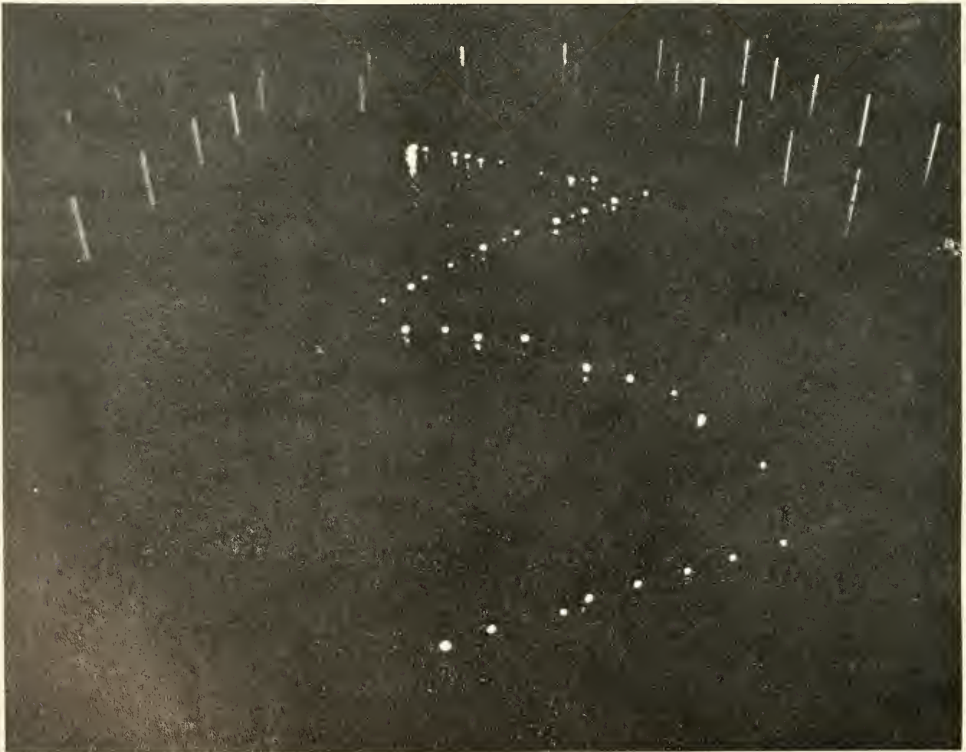
Figure 7   Blinker trail, showing path of nurse shark homing on a stimulus source (TMAO and betaine) by true gradient searching, or klinotaxis. The stimulus source is located at the far end of the observation pen, at the bright spot caused by the shark staying in that vicinity, once the stimulus is reached.
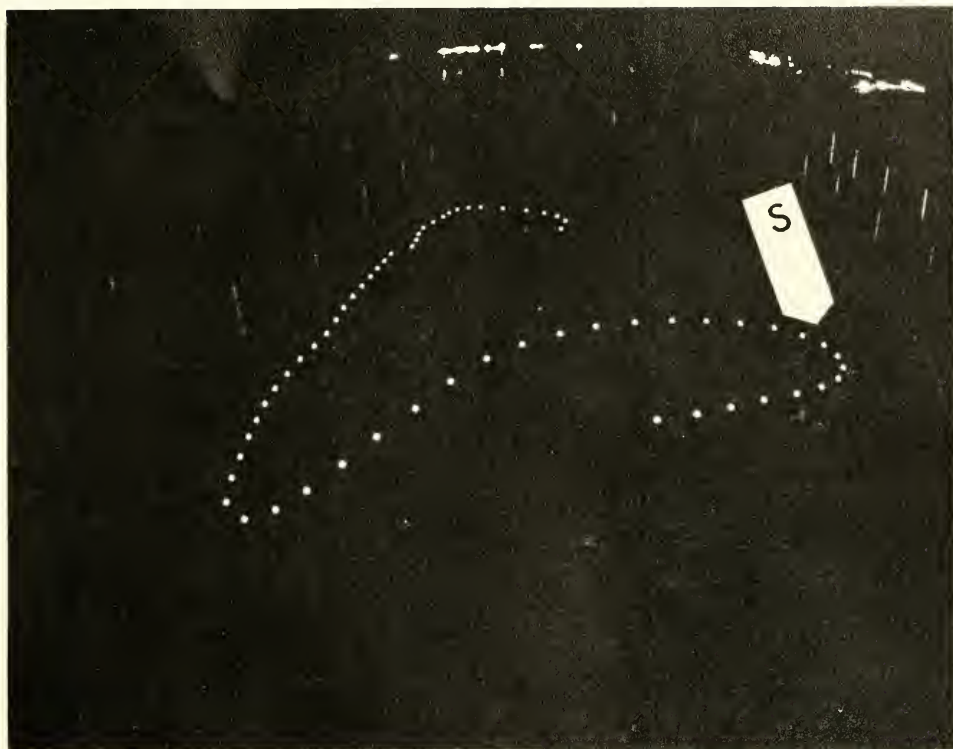
Figure 8   Blinker trail of a lemon shark responding to amine stimulus. Olfactory corridor from stimulus in the far right corner of observation pen is contacted at point S. Shark then swims into strongest current along left side of pen, starting to circle in a different corner from the true origin of the stimulus.

(e.g., repeated biting at objects in the water near the stimulus release point, milling about near the release point with aggressive behavior toward other sharks or fishes) is indicated by "++." Initial negative responses to the stimulus (turning away from the olfactory corridor, head shaking, and moving away from the stimulus) is indicated by a "0" in the behavioral response column.

It was particularly difficult to be sure of continued negative responses during the 15 min of testing any single chemical stimulus; sharks that turned away from the stimulus might simply never encounter it again, or they might shift to weak positive responses, suggesting that they were returning to investigate the stimulus after an initial turning away; the latter responses are indicated in the table by "0 → +."

Concentrations tested are given in a range from those maximal concentrations initially introduced into the test area, to the dilutions obtained by dilution in the laboratory or by tidal flow. In the large pen, dilutions of 100 000 or more were found across the length of the test enclosure at some stages of tide.
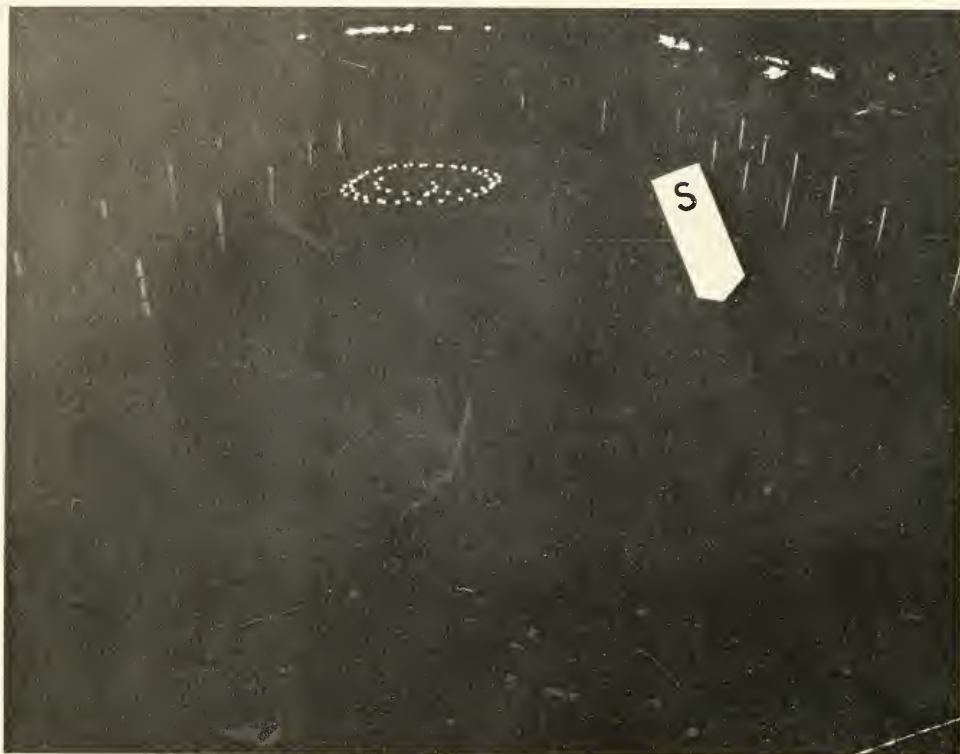
Figure 9   Blinker trail of lemon shark, continuing record shown in Figure 8. Shark circles repeatedly in corner of pen where strongest current enters, despite the fact that the stimulus comes from opposite corner and moves along olfactory corridor labeled S. Record covers approximately 1 min of time.

It was not the aim of these studies to establish absolute thresholds for the effectiveness of the chemical stimuli tested. To a considerable extent, judgments of "thresholds" necessarily depend on the refinements of criteria in judging EEG effects or behavioral changes. However, the results with the most effective stimuli indicate that the *range* of thresholds for those compounds lies somewhere between 10^{-6} and 10^{-9} molar concentrations, under the test conditions.

Summary of EEG/Behavioral Results

The main reason for the survey of various categories of pure chemicals was to ascertain whether the chemoreceptors were responding to a narrow range of ionic or molecular structures, which might make it possible to characterize the actual receptor sites involved. The experimental system was simplified somewhat by narrowing the receptors to olfactory receptors in the nasal sac, for all the responses noted were eliminated when a local anesthetic (procaine) was introduced into the nasal sac before testing; removal of both barbels from experimental nurse sharks, however, did not diminish their re-

Table 1. EEG and behavioral responses to pure chemicals and extracts added to sea water.

Compound	Range of concentrations (molar) tested	EEG response	Behavioral response
Electrolytes			
NaCl	$10^{-1} \rightarrow 10^{-7}$	+	+
NH_4Cl	$10^{-1} \rightarrow 10^{-7}$	+	+
Kcl	$10^{-1} \rightarrow 10^{-7}$	+	+
$CaCl_2$	$10^{-1} \rightarrow 10^{-7}$	+	+
$MgCl_2$	$10^{-1} \rightarrow 10^{-7}$	+	+
LiCl	$10^{-1} \rightarrow 10^{-7}$	+	+
KI	$10^{-1} \rightarrow 10^{-7}$	+	+
KNO_3	$10^{-1} \rightarrow 10^{-7}$	+	+
KBr	$10^{-1} \rightarrow 10^{-7}$	+	+
KF	$10^{-1} \rightarrow 10^{-7}$	—	+
$HgCl_2$	$10^{-1} \rightarrow 10^{-7}$	— (?)	+
$CuSO_4$	$10^{-1} \rightarrow 10^{-7}$	— (?)	+
Artificial seawater	(N.A.)	—	+
2 × seawater	(N.A.)	+ (?)	+
0.5 seawater	(N.A.)	—	+
Carbohydrates			
Glucose	$10^{-3} \rightarrow 10^{-9}$	—	+ (?)
D-Arabinose	$10^{-3} \rightarrow 10^{-9}$	—	—
L-Arabinose	$10^{-3} \rightarrow 10^{-9}$	—	+ (?)
Glycogen suspension	(N.A.)	—	—
Amino acids and amines			
Glycine	$10^{-3} \rightarrow 10^{-9}$	+	++
Glutamic acid	$10^{-3} \rightarrow 10^{-9}$	+	++
Cysteine	$10^{-3} \rightarrow 10^{-9}$	+	+
D-Serine	$10^{-3} \rightarrow 10^{-9}$	+	+
L-Serine	$10^{-3} \rightarrow 10^{-9}$	+	+
D-Methionine	$10^{-3} \rightarrow 10^{-9}$	+	+
L-Methionine	$10^{-3} \rightarrow 10^{-9}$	+	+

Note: "+" indicates consistent EEG changes or positive behavioral responses. "—" indicates lack of detected responses. (N.T.) designates not tested. (N.A.) designates measurement not applicable. For further details consult text.

Table 1. EEG and behavioral responses to pure chemicals and extracts added to sea water.—Continued

Compound	Range of concentrations (molar) tested	EEG response	Behavioral response
Betaine	$10^{-3} \rightarrow 10^{-9}$	++	++
Trimethyl amine	$10^{-3} \rightarrow 10^{-9}$	++	++
Trimethyl amine oxide	$10^{-3} \rightarrow 10^{-9}$	++	++
Lipids (emulsions)			
Stearic acid	(N.A.)	+ (?)	—
Linoleic acid	(N.A.)	+ (?)	$0 \rightarrow$ —
Glycerol	(N.A.)	—	—
Cod liver oil	(N.A.)	—	—
Tuna liver oil	(N.A.)	—	—
Polyhydric alcohols			
Sorbitol	$10^{-3} \rightarrow 10^{-9}$	—	—
Dulcitol	$10^{-3} \rightarrow 10^{-9}$	—	—
Mannitol	$10^{-3} \rightarrow 10^{-9}$	—	—
Inositol	$10^{-3} \rightarrow 10^{-9}$	—	—
Purified blood fractions			
Hemoglobin—human (2 × crystallized)	(N.A.)	(N.T.)	+
Hemoglobin—bovine (2 × crystallized)	(N.A.)	(N.T.)	+
Albumin—human (crystallized)	(N.A.)	(N.T.)	+
Albumin—bovine (crystallized)	(N.A.)	(N.T.)	+
Beta globulin Cohen fraction III	(N.A.)	(N.T.)	—
Beta lipoprotein Cohen fraction III-0	(N.A.)	(N.T.)	—

Note: "+" indicates consistent EEG changes or positive behavioral responses. "—" indicates lack of detected responses. (N.T.) designates not tested. (N.A.) designates measurement not applicable. For further details consult text.

Table 1. EEG and behavioral responses to pure chemicals and extracts added to sea water.—Continued

Compound	Range of concentrations (molar) tested	EEG response	Behavioral response
Miscellaneous extracts			
Holothurin (extract of Cuvier's gland of *Actinopyga*)	(N.A.)	+	0 → —
Aplysia "ink" secretion	(N.A.)	—	0 → —

Note: "+" indicates consistent EEG changes or positive behavioral responses. "—" in-
dicates lack of detected responses. (N.T.) designates not tested. (N.A.) designates
measurement not applicable. For further details consult text.

sponses in these tests. The effect of the anesthetic in the nasal sacs made it clear that gustatory receptors were not significantly influencing the responses being studied, and it was assumed that other chemoreceptors must come into play later, when ingested material is very near or within the mouth.

Electrolytes—Two series of electrolytes were tested in what has become a standard approach to analyzing the effectiveness of ions on chemoreceptors. A series of different cations were tested, in combination with one anion (Cl^-); different anions were then tested in combination with the same cation (K^+). Chemoreceptors of mammals and insects have been shown to be particularly sensitive to cation effects, with the stimulating effectiveness of various cations roughly parallel to their effective electrostatic field strengths (Hodgson 1974). However, the sharks, while showing obvious awareness of the ionic stimuli, did not demonstrate differential sensitivities that could be detected by these methods. Nor did they give sustained behavioral responses to any of the electrolytes.

The great sensitivity of the shark's olfactory system is illustrated strikingly by the discrimination between artificial seawater (containing the 10 major electrolytes of seawater) and the natural seawater of the test area. (This may not be entirely on the basis of electrolytes, of course, since the artificial seawater would lack other compounds dissolved in natural seawater.)

EEG responses of $HgCl_2$ and $CuSO_4$ were weak and of short duration, probably a result of the toxic effects of these metal ions on the olfactory epithelium (Hodgson 1965, Tester 1963). In behavioral tests in the large enclosure, there were no sustained reactions, either positive or negative, to $HgCl_2$ or $CuSO_4$. This could be the result of the quick rinsing of the nasal sacs during normal swimming, rather than the toxic effects observed in tests in more confined quarters.

The interpretations of results of tests with electrolytes has been discussed in more detail by Hodgson, Mathewson, and Gilbert (1967). It appears unlikely that ions constitute major cues to feeding in sharks, but it is entirely possible that sharks might orient toward or away from hypersaline (or hyposaline) waters, using reactions mediated by their chemoreceptors.

Carbohydrates—The series of carbohydrates tested has been particularly revealing about sugar detection mechanisms of mammals and insects. In both groups, one category of chemoreception can be traced to particular molecular configurations of carbohydrates (Hodgson 1974). Evidently the situation is quite different with sharks, since EEG responses were absent and behavioral responses were considered questionable.

Amino acids and amines—Protein breakdown products and related compounds have long been known to stimulate chemoreceptors in a variety of marine animals. Among invertebrates, studies that demonstrate this range from experiments on carnivorous coelenterates (Laverack 1968) to experiments on many marine arthropods (Case and Gwilliam 1961, Levandowsky and Hodgson 1965). Hara (1973, 1975, 1976) has demonstrated the high stimulatory effectiveness of certain amino acids for olfactory receptors of teleost fishes. Accordingly, five amino acids and three tertiary amines were chosen to determine whether sharks might have some of the same sensitivities and responses.

As shown in Table 1, this was one of the most stimulating groups of pure chemicals tested. Both EEG and behavioral responses were striking and prolonged. Figures 5 and 6 illustrate EEG responses, and Figures 7, 8, and 9 show typical orientation responses by nurse and lemon sharks.

Glycine and glutamic acid were the most effective of the pure amino acids tested. Betaine, trimethylamine (TMA), and trimethylamine oxide (TMAO), all breakdown products in or from tissues or excreta of fish, elicit particularly strong responses under the test conditions. This parallels many of the results with arthropods and teleost fishes and raises an interesting evolutionary question, for fishes at least. That is, do certain specialized olfactory cells of these animals derive from a single ancestral cell type in evolution? The adaptive value of this sensitivity to protein constituents and breakdown products for predatory carnivores is obvious.

Lipids and related compounds—Stearic acid was tested as an example of a saturated fatty acid and linoleic acid as an example of an unsaturated fatty acid. Because of its special importance in conjugating with fatty acids to form true fats, glycerol was also tested. (Solubility characteristics dictated that the fatty acids were tested as emulsions.) The polyhydroxyl alcohols (sorbitol, dulcitol, mannitol, and inositol) were tested because they had provided insight regarding mechanisms of other chemosensory systems, and because inositol is useful for identifying chemoreceptors responding to ring-structured, rather than straight chain, molecules. Emulsions of cod and tuna liver oils were used to check the commonly held belief that sharks prefer prey and meat with higher oil concentrations.

EEG records showed slight responses during tests with glycerol and the fatty acids, but there were no consistent behavioral changes with these compounds as stimuli. The emulsions were nonstimulating, with one curious exception. A lemon shark happened to approach the tygon tube through which linoleic acid emulsion was being introduced; a violent shaking of the shark's head was observed, and the animal quickly swerved and swam away from the stimulus source and olfactory corridor. This incident is mentioned because isolated episodes of violent aversive reactions do occur from time to time, and it is tempting to interpret them as indicating the repellent capacities of the stimuli. However, they cannot be repeated consistently. Rather than repellent actions, most such events observed in these experiments were more likely the result of startle responses or transient irritation effects from the introduction of high concentrations of the emulsions into the nasal arcs. The absence of responses to the fish oil extracts supports the view that these oils are not major chemical cues in prey-seeking behavior.

Purified blood fractions—Although attraction of sharks to fresh blood in the water has been observed many times, the results of controlled tests with stored blood, usually outdated supplies from hospital blood banks, have been ambiguous. Sometimes such tests elicit oriented approaches by the experimental sharks, sometimes not. Moreover, the obvious next step of testing carefully refined components of whole blood, to determine the attractive chemicals more specifically, was handicapped by the unavailability of most suitable fractions for testing, and by the high costs of the few that were available. The latter possibility has recently become more feasible through the availability of commercial supplies of purified blood components from nearly a dozen mammalian species.

Taking advantage of the purified blood fractions available, we chose a test series to include major fractions of human and bovine blood components, with the ultimate choices depending on relative costs and availability at the time of testing. Because the behavioral thresholds were significantly lower than EEG responses, the blood fractions have thus far been tested only for behavioral responses of free-swimming sharks. The fractions listed in Table 1 were introduced in the test enclosure as concentrates freshly obtained from commercial suppliers.

At least brief awareness responses were obtained from both nurse and lemon sharks contacting the olfactory corridors formed by all the blood fractions. The human and bovine hemoglobins (2X crystallized) elicited clear orientation and approach reactions, with significant increases in the swimming speeds of responding lemon sharks. Less stimulating, but still eliciting positive orientation and approaches, were the human and bovine albumen (crystallized) fractions. Globulin (beta globulin, Cohen fraction III) did not elicit sustained orientation responses.

These results are encouraging in that they indicate the varied effectiveness of different purified blood fractions. However, they are not yet susceptible to more detailed interpretation. It seems likely that breakdown products in seawater, perhaps amino acids, are the essential stimuli involved. It is also

tempting to speculate that the relative ineffectiveness of globulin extracts is related to their higher molecular weights and relative insolubility. Much more needs to be known about the fates of these purified fractions in seawater, and more quantitative comparisons of electrophysiological responses such as the EEGs should be obtained before additional conclusions can be drawn from the studies on blood fractions.

Chemical stimuli eliciting negative responses—A large amount of effort has been invested in the search for chemicals acting as powerful and consistent repellents as sharks. Rather than reviewing those chemical compounds and past studies in detail, it can be said, in summary, that no ideal repellents have been found. Indeed, there are persuasive theoretical reasons for believing that repellents capable of practically deterring attacking sharks do not exist. This does not mean, however, that no chemicals repel sharks in their normal environments. The whole question of shark repellents, as well as new approaches to their study, is considered in more detail below.

MECHANISMS OF ORIENTATION—INTEGRATION OF STIMULI

The large, 40-by-80-ft (12.3 by 24.6 m) observation and test pens used in these behavioral studies on sharks provided a good opportunity to analyze further the mechanisms used by nurse and lemon sharks in locating sources of chemical stimuli. These mechanisms proved to differ in the two species.

Contributing to the analysis of orientation mechanisms was the fact that tidal flow was at different rates on the two sides of the observation pen. This made it possible to introduce a chemical stimulus on the strongest current flow or in an area of weak flow. Consequently, swimming patterns of stimulated sharks could be studied as a function of the direction and rate of water flow, in addition to the variables of the chemical stimuli. Photographs of locomotor tracks were made with motion pictures in the daylight hours, and with open-shutter photos of towed electronic flash units at night (Hodgson and Mathewson 1971, Mathewson and Hodgson 1972).

It was found that nurse sharks homed in on a stimulus source via an S-shaped track, using true gradient searching, or klinotaxis (Figure 7). The extent of the side-to-side excursions decreased with the number of the test in a repetitive sequence of trials, suggesting that some learning was involved during repeated testing. In addition to the decreased sideways excursions, under repeated testing the nurse sharks showed increased speed and efficiency in approaching a stimulus.

Although the nurse sharks, unlike the lemon sharks, did not move into the strongest currents following chemical stimulation, there was no part of the observation pen which completely lacked some current flow. In a subsequent study on mechanisms of localizing chemical stimuli, Kleerekoper and Gruber (1975) found that in stagnant water only generalized localization occurs; hence, some movement of flowing water "provides the direction vector for precise localization" even by nurse sharks (Kleerekoper 1978).

With lemon sharks, the influence of water currents was found to be greater. Once stimulated by a chemical, lemon sharks swam upstream in the strongest water current passing through the observation pen at the time. They did this regardless of whether the behavior brought them closer to the source of chemical stimulation. Figures 8 and 9 illustrate one such response, in which a lemon shark circles repeatedly in a corner of the pen where the strongest current enters; the chemical stimulus, which prompted the reaction, remains all the while in a slower current about 10 m away in the pen. The reactions of the stimulated lemon sharks, therefore, are dominated by a reaction to water currents, or rheotaxis. Normally, this would bring the lemon shark near to food material or prey, at which time other cues (e.g., visual or electrical) might come into play. Only in the experimental situation is the true nature of the lemon shark's rheotaxis revealed.

It has been postulated (Mathewson and Hodgson 1972) that the use of chemical stimuli primarily to trigger a rheotaxis, as in *Negaprion*, makes fewer demands on the chemosensory receptor system than does a purely klinotaxic response. Stimulation by a minimum threshold number of molecules could suffice to trigger the rheotaxis, without the necessity for "comparing" or "balancing" the number of stimulating molecules hitting chemoreceptors on the two sides of the head. Such a rheotaxis-release mechanism had been postulated by Kleerekoper, and it is known to be operative in many other marine animals (Kleerekoper 1978).

It remained to be determined whether the observed orientation mechanisms were related to the final stages of feeding, when visual stimuli become very important in guiding biting responses and other components of attacks upon prey. Convincing evidence that these patterns of orientation can lead to typical biting, even "frenzied" feeding behavior, came from six cases in which combinations of amines and amino acids elicited strong feeding behaviors, even though the chemical solutions provided no visual cues for close-range attack. In all these cases, the sharks began biting (lemon sharks) or sucking (nurse sharks) when within 2 m of the stimulus source. This feeding behavior appeared to be directed toward whatever small objects the shark encountered in the water when near the highest concentrations of chemical stimuli—bubbles on the water surface, small twigs or grass blades being carried past in the water current, etc. The role of vision in these reactions was especially clear in *Negaprion*, as observed and photographed from the underwater observation cage near the stimulus source. As a lemon shark neared the outlet tube for chemical stimuli, its eyes could be seen to move whenever it swam past small objects in the water; the correlation of eye movements and slight shifts in swimming directions, related to whatever floating objects were visible, were quite different from the responses of the same sharks in other areas of the test pens. The frontispiece of this book provides an illustration of such a response. It shows a lemon shark "attacking" some air bubbles that were originally limited to the water surface, about 2 m from the source of a stimulus stream consisting of 0.1 M TMAO mixed with 0.1 M glycine. The shark's biting and side-to-side head movements during the initial attack produced more bubbles, and the shark

intensified its biting attempts, persisting for 40 s before swimming out of the olfactory corridor.

Open Sea Tests

Further evidence of the efficacy of tertiary amine and amino acid in mixtures for eliciting feeding behavior came from open sea tests carried out at the Lerner Marine Laboratory, Bimini. An underwater television camera, at a depth of 65 ft (20 m), near the edge of the Gulf Stream, allowed the behavior of sharks to be studied without any intrusion of observers. All the sharks were wild and had never been confined.

In these experiments, chemical stimuli flowed slowly from a perforated source bottle. The fish and sharks that appeared before the camera, in the vicinity of the chemical stimulus, could be compared with the fauna observed in the same area during extended periods of study in the absence of any added chemical stimulation, both before and after these experiments.

When mixtures of tertiary amines and amino acids were first released at the television study site, a sequence of fish species swam to the area, usually culminating in some of the larger species, such as the Nassau grouper (*Epinephelus striatus*). Typically, all teleost fishes withdrew from the area 30–90 s before any sharks appeared, thus providing a convenient signal for the observer monitoring the TV screen in the laboratory (Figure 10).

Lemon sharks, nurse sharks, and sharp-nosed sharks (*Rhizoprionodon terraenovae*) were the species most commonly attracted to the amine-amino acid mixtures. Lemon sharks invariably approached by moving upcurrent, whatever the direction of flow at the time of the test. Curiously, they were never observed to bite at the stimulus source bottle; nor did they linger in the area more than a few seconds, but always continued their swimming against the current.

Both nurse sharks and sharp-nosed sharks swam into the area from various directions, but in most cases (12 out of 17 positive identifications) arrived from a direction that was downcurrent at the time. Consequently, it was concluded that some rheotaxic cues were used by these species, too, in orienting toward a chemical stimulus. On arriving near the source bottle, nurse and sharp-nosed sharks circled the bottle, occasionally settling down to lie immobile with their snouts pressed against the concrete block supporting it. There was no doubt that these species made a very precise localization of the source of chemical stimuli. However, it was not certain, because of the size and the lighting of the TV image, whether jaw movements occurred in these sharks when they were near the bottle.

In an entirely different type of open sea test, mixtures of TMAO and glycine were allowed to seep from a perforated source bottle that was tethered in a narrow gully, cutting through a small fringing reef. The gully carried currents up to 0.7 knot during tidal flow between the shallow lagoon and an area seaward of the reef. The area just outside this fringing reef was known to be a particularly good site for shark collecting near Bimini. Consequently, the chemical stimuli were moving out from a well-defined olfac-
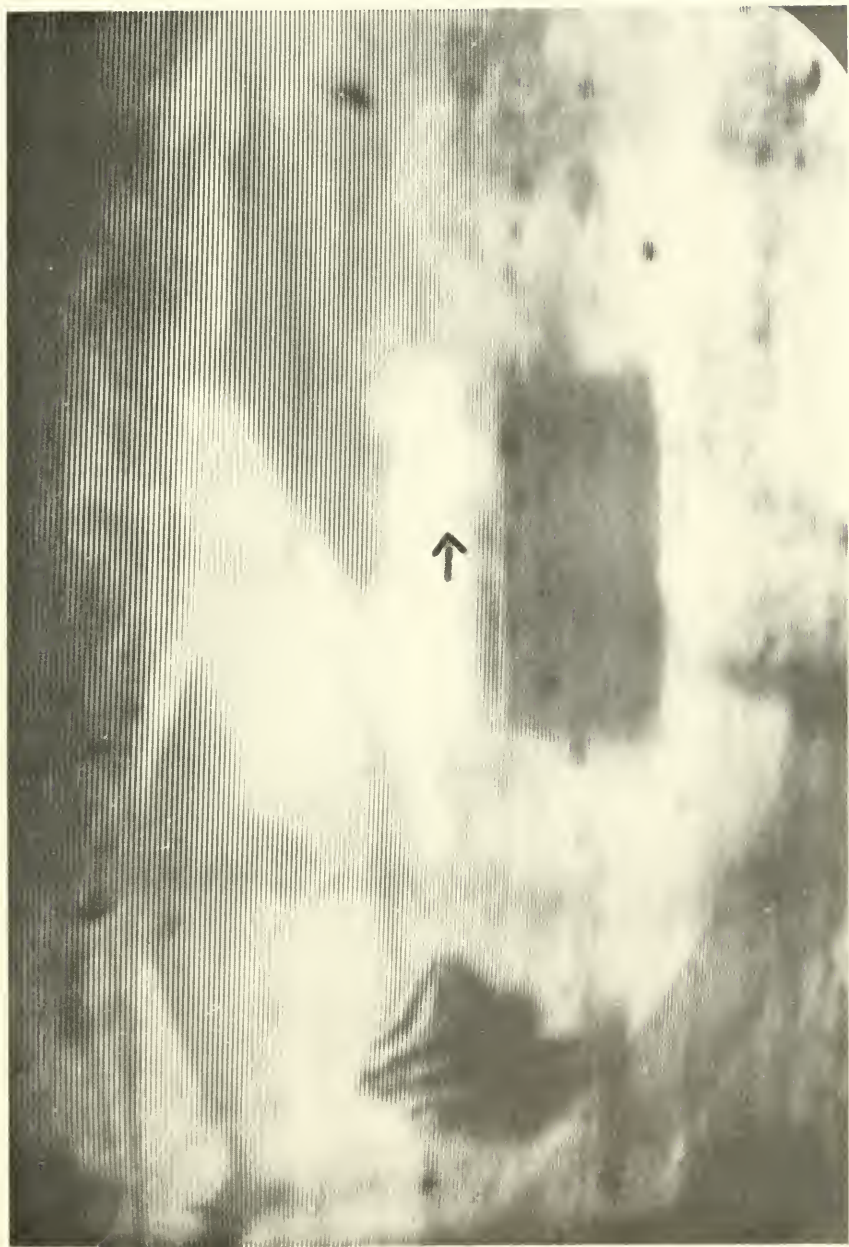
Figure 10 Two sharp-nosed sharks circle source of amine-amino acid mixture, at 65-ft (20 m) depth, on front of underwater television camera. A Nassau grouper is seen at the left. Arrow points to stimulus source in concrete block. Photographed from TV monitor screen.

tory corridor in the gully, during outflowing tides, and spreading out over a wide area of shallow water (6–20 ft; 1.8–6.1 m), where sharks might be expected to encounter the stimuli.

In four tests, the TMAO-glycine mixture attracted two lemon sharks and a large ray (probably *Dasyatis americana*) to the vicinity of the channel through the reef. One lemon shark swam against the current, entered the gully, approached the stimulus source bottle, and vigorously bit the bottle (Figures 11 and 12). It is possible that the movements of the tethered bottle in the current supplied visual stimuli that summed with the chemical stimuli to elicit the biting responses. The stimulus bottle at the deep water television site had been firmly anchored inside a concrete block, and was immobile.

The results of the open sea tests are compatible with the conclusions reached from experiments in the hydrodynamic tunnel and in the large observation pens. The role of rheotaxis in the orientation responses was confirmed, and the adequacy of mixtures of tertiary amines and amino acids for releasing feeding behavior under certain conditions was further supported. The data suggest that moving visual stimuli may be important releasers of biting activity in the final stages of orientation behavior, when sharks are surrounded by higher concentrations of effective stimuli in the water. These observations are also compatible with the conclusions of
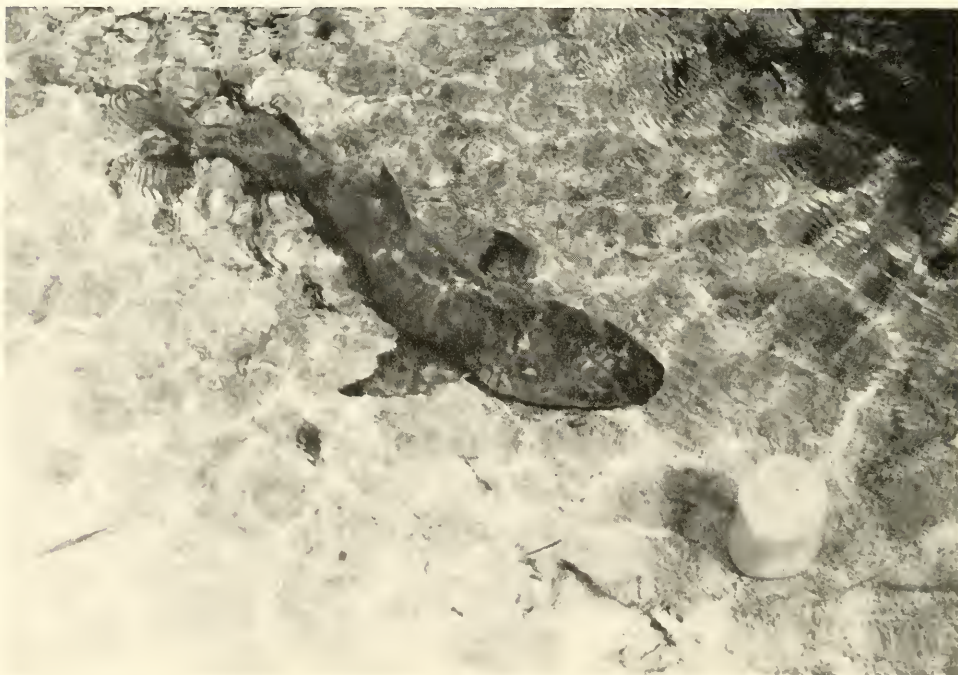
Figure 11   Lemon shark approaches TMAO-glycine mixture being released from bottle tethered in a channel through fringing reef. See text for details.

Figure 12   Lemon shark biting source bottle of TMAO-glycine mixture in reef channel.

Hobson (1963), based on his study of several shark species in the Pacific. In most cases he found that baits were approached from downstream but that actual feeding required additional stimulation, such as visual cues. Kleerekoper (1978) discusses, elsewhere in this volume, other variables (light, "handedness" of sharks, etc.) that can modify the locomotor activities of sharks. If, to all these important variables integrated by the CNS and controlling locomotor patterns, the individual variable of learned experience by the shark is added, it is clear that what might appear to be a rather simple stimulus-response behavior pattern is actually considerably more subtle and is delicately adjusted to many environmental variables.

THE TRANSDUCTION MECHANISMS OF CHEMORECEPTORS

A central consideration in any physiological analysis of chemosensory functions is the search for the basic transduction mechanisms, by means of which the chemical energy is, in effect, transformed into the electrical potential across the stimulated chemoreceptor membrane. Only a beginning has been made in approaching this level of analysis with the chemoreceptors of elasmobranchs. At present, it appears that at least several types of chemical

stimuli are highly effective in stimulating olfactory receptors of sharks—the most studied examples. Consequently, at least several different types of transduction mechanisms might be involved. These might involve specialized receptor sites for transduction of the following chemical stimuli:

1. Electrolytes
2. Tertiary amines and/or amino acids
3. Certain blood factors or their breakdown products:
   (a) hemoglobin
   (b) albumen fractions.

There may be overlaps in this list (e.g., breakdown products of blood fractions and amino acids), and the list is almost certainly incomplete.

Now that the effectiveness of at least these types of compounds have been shown through both EEG and behavioral analyses, it should be possible to conduct more extensive comparisons of stimulus effectiveness, using more examples from each category of stimuli and standardized electrophysiological recording methods. For example, Hara (1976) has shown, by comparing the effects of many amino acids and their isomers on electrical responses of olfactory bulbs in rainbow trout, that it is possible to draw more precise conclusions about the structure activity relationships of amino acids in fish olfaction. Present results with sharks are compatible with Hara's conclusion that simple, short, straight-chained amino acids are effective chemical stimulants, but nothing can yet be concluded about the effects of side chains attached to these amino acids, the influence of various isomers, etc. Such studies will be required to characterize the hypothetical receptor sites of the olfactory chemoreceptors of elasmobranchs.

Another basic question is whether differently specialized receptor membranes may be present on different types of chemoreceptor cells within the olfactory epithelium. Pyatkina (1974) has postulated that this may be so for the three forms of receptor cells found in the olfactory epithelium of the sturgeon, and Kleerekoper (1978) has discussed other similar examples.

EXPERIMENTS WITH RADIOACTIVE STIMULI

The exact sites of effects by various chemical stimuli and the fates of the stimulating ions or molecules, once they arrive at the chemoreceptor membranes, and matters of concern in all studies on chemoreceptors. How strong is the link between stimulus and receptor site? How long is the link maintained? Is the stimulus metabolized by the receptor cell or dispersed by some mechanism external to the receptor? These and similar questions are beginning to be answered for some chemosensory systems by the use of radioactively labeled chemical stimuli known to be highly effective in the particular systems studied.

Binding of tracer-labeled pheromones onto chemoreceptor sites of insects has been recorded. A natural "rinsing" mechanism of some insect chemoreceptors has also been noted following the movement of tritiated water

flowing over the chemoreceptor cells (Hodgson 1967). For studies on the fate of a known chemical stimulant of the shark's olfactory system, tritium-labeled d-l glutamic acid was chosen.

In experiments conducted in collaboration with Dr. Arland Carsten of the Brookhaven National Laboratory, the labeled glutamic acid was injected into a seawater stream perfusing the nasal sac of an anesthetized nurse or lemon shark. When a bolus of labeled stimulus (2 ml of solution, containing 200 μCi of radioactivity in glutamic acid) was introduced in the nasal sac, it was found that the pattern of flow through the olfactory sac was no different than the flow pattern of a dye through the same system (Figure 13). Radioactivity in the outflowing perfusate was measured in samples taken every 5 s during perfusion, and counted in a scintillation counter. The counts rose rapidly after the stimulus was introduced, and fell to zero after 150 to 200 s. (The exact shape of the curves varied according to the anatomy of the olfactory sac in each shark; in the example illustrated in Figure 13, any counts of less than 10^3 are not significant, since the larger amounts approach 1.56×10^7. Also, in a semilog plot, the slight differences at the initial and concluding low ends of the curve are greatly magnified.)

The lack of tracer activity in the outflow of perfusate after the bolus of labeled stimulus had passed through the nasal sac suggests that seawater flow through the sac is not significantly impeded by the internal folds of the sac, intricate as they are (Plate II). Another possibility is that any stimulus bound to the receptor membranes remains there, possibly being metabolized or otherwise disposed of in the olfactory epithelia. To check the latter possibility, solutions of radioactively labeled glutamic acid were placed directly in the olfactory sac and allowed to remain there for periods of up to 5 min, after which the sac was flushed with a minimal amount of seawater and removed. The tissue was fixed and sectioned, and autoradiographs were prepared. When the autoradiographs were evaluated, no labeled material was seen in the convoluted membranes containing the chemosensory cells. Plate II shows one of the sections, stained with hematoxylin, from this series of autoradiographs. It is remarkable that none of the labeled stimulant, even by chance, remained in or on the olfactory epithelium; the only radioactivity detected was in a few spots in spaces between epithelial folds, not bound to any tissue.

What these results evidently mean is that there is no prolonged binding of the stimulus to any part of the receptor membranes—at least so far as glutamic acid is concerned. Also, the flushing mechanisms of the olfactory sacs are impressively efficient, with the flow pattern through the sac approaching that of flow through an unobstructed orifice. Such a flow pattern has obvious advantages in facilitating moment-to-moment comparisons of input to the two nasal sacs, and in preventing lingering aftereffects of stimulation. Since it is possible that there may be more than one chemosensory mechanism or site, it remains to be seen whether similar flow patterns and lack of detectable binding will be found in tests with other types of labeled and effective chemical stimulants.
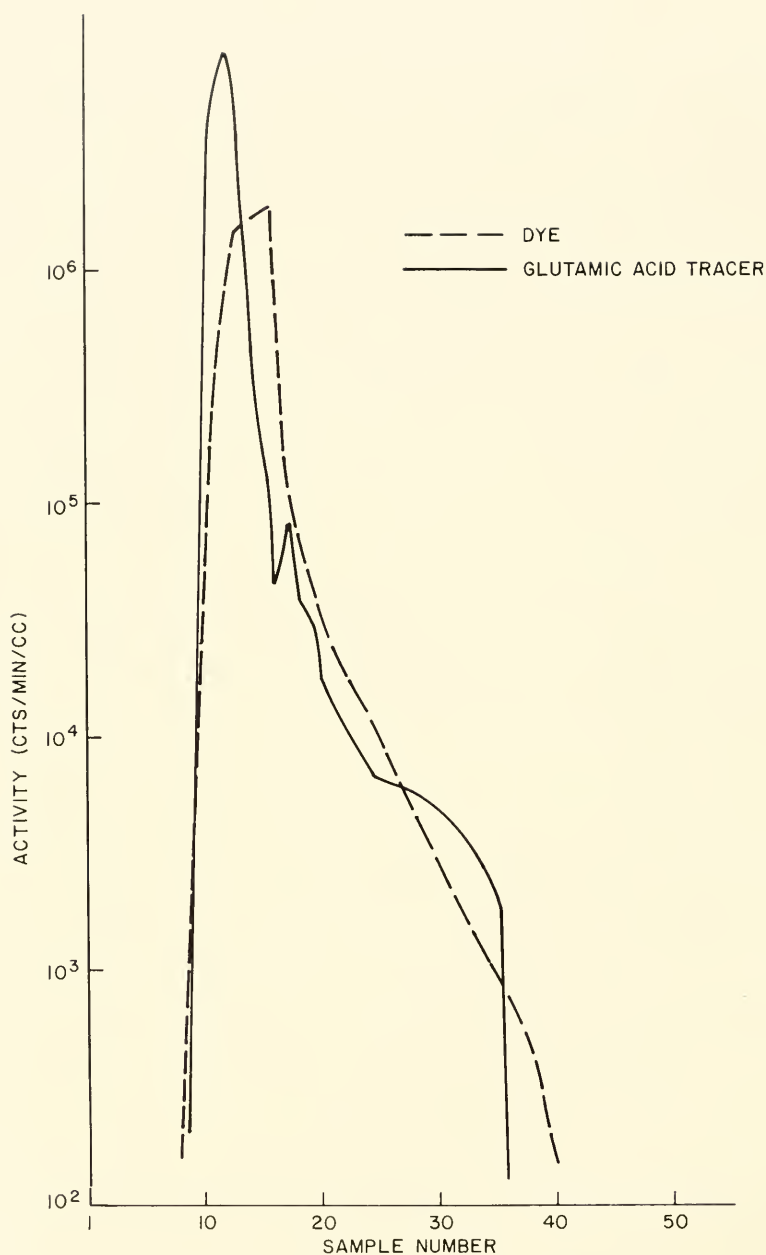
Figure 13 Movement of tritium-labeled d-l glutamic acid, compared with movement of inert dye through the olfactory sac of a lemon shark. The tracer activity (solid line) is plotted on a semilog scale and the dye densities (dashed line) on a different scale, to facilitate comparisons of the time course of travel of each substance through the sac.

Plate II Cross section through olfactory epithelium taken from olfactory sac of a lemon shark; hematoxylin stain. This specimen is from a series of autoradiographs, and the absence of tracer-labeled material (which would appear black) in the wavy open channels between folds of the olfactory mucosa indicates complete flushing of stimulus from the olfactory system.

THE SEARCH FOR CHEMICAL REPELLENTS

An immediate and practical motivation for much research on the chemo-sensory systems of sharks has been the desire to identify chemical repellents. What may seem to be an obvious objective, even a relatively simple one, turns out to be anything but simple. The key difficulty arises from what is meant by the term "repellent." It may be interpreted, most simply, as any chemical that lessens the probability of a shark's ingesting some normal food substance. On the other hand, the word may be taken to mean a chemical capable of deterring biting, or any other form of attack, by a shark already in a feeding frenzy and behaving aggressively toward virtually anything seen in its immediate vicinity. In the latter case, the repellent would have to be much more than a compound that imparts an unpalatable flavor to a poten-tial food; it would have to incapacitate an attacking shark, through some powerful pharmacological or toxic action. Considerable confusion has arisen from discussion of repellents without specifying what type of repellent action is being studied or sought.

Early evidence of the simpler types of repellents, decreasing food inges-tion, was obtained from several classic studies, notably that by von Uexkull (1895). He demonstrated that quinine prevented ingestion of fish meat, and that sharks that took quinine-mixed baits into their mouths promptly spit them out; after the quinine was washed out of the bait, the meat was readily ingested. Von Uexkull was also among the first to demonstrate a natural repellent, one produced by the marine gastropod *Aplysia*. Sharks excited by fish baits were observed to deviate from their usual behavior of ignoring these marine slugs; when taken into the mouth of a shark, the *Aplysia* was promptly spat out.

A systematic search for protective shark repellents was begun in the 1940s, after many reports of shark attacks on American servicemen from aircraft and ships downed in the tropical Atlantic and Pacific. The first experiments were conducted at the Woods Hole Oceanographic Institution, using the smooth dogfish, *Mustelus canis*. Unfortunately, the dogfish was not deterred from eating by any of the candidate repellents tested: systemic poisons, chemical warfare gases, ink clouds, etc. Gilbert and Springer (1963), Tester (1963), and Gilbert and Gilbert (1973) have reviewed many of the experiments aimed at the discovery of repellents during that period.

The following list, incomplete, indicates at least part of the range of com-pounds and extracts tested in the hope that one or another of them might repel sharks: acetylcholine, amino acids, ammonium hydroxide, choline chloride, cortisone acetate, creatinine, diallyl maleate, diallyl phthalate, furfural, histamine, human sweat, human urine, nicotine, and phenylacetate salts. Suffice it to say that none of these gave any evidence of repellent properties.

The repellent actually developed, and still available, was called Shark Chaser, a mixture of 20% copper acetate and 80% nigrosine dye in a water-soluble wax cake, packaged in a plastic envelope. The rationale for the choices of ingredients has been reviewed by Gilbert and Gilbert (1973), and derives from observations upon the reactions of sharks to breakdown

products of rotting shark flesh and to clouds of dye. Since the significant breakdown products of shark meat were shown to contain large quantities of acetic acid, and copper ions appeared to have at least sporadic repellent effects, the combination of copper acetate was chosen as the best approximation of a repellant known at the time. The dye cloud, which at least temporarily inhibited approaches by some large sharks under some conditions, was even more important as a psychological boost to the users. The limitations of Shark Chaser are demonstrated in Plate I, which illustrates a positive response to the "chaser" when the dye was being used to map olfactory corridors in a large observation pen.

It is important to note that a repellent may have some useful functions, even though it does not work against sharks in a feeding frenzy or produce long-lasting effects against all species. Despite a research crash program, and tacit admission by the producers that Shark Chaser was *not* "the ultimate repellent," experiments during the 1960s produced no strikingly better alternative. In fact, studies on the kinetics of responses by sharks to water-borne drugs strongly indicated that the most powerful repellents, capable of incapacitating attacking sharks, are unlikely to be found. This conclusion was based, in large part, on experiments by Baldridge (1969*a*) concerning the actions of incapacitating drugs on lemon sharks.

Baldridge determined the minimum drug concentrations in seawater necessary for producing states of excitation, depression, and narcosis or incapacitation in sharks. The effects produced by drugs such as quinaldine were found to involve mass-action effects on a number of systems in which drug—receptor combinations are relatively weak and highly reversible. In practice, very high concentrations of such drugs would have to be built up and sustained in a shark's body to produce significant behavioral alterations. Strychnine and hydrocyanic acid are more selective and specific in their actions, but still would be unlikely to incapacitate sharks in open water without reaching concentrations that would be impractical to deliver and would be dangerous for human beings in the area (Baldridge 1969*b*).

The general impact of the studies in the 1960s was to turn investigations away from unrealistic hopes of finding "ultimate" repellents, capable of incapacitating sharks. Attention has been shifted to chemicals that might render an environment uninviting, decrease the palatability of potential prey, or (if specific chemoreceptor sites could be characterized) compete with normal stimuli at the transduction sites to block the effectiveness of sensory cues. The experiments with tracer-labeled stimuli suggest that the latter approaches will not be easy, at least with amino acids, since any binding of stimulating molecules appears to be weak and quickly reversed, and the normal rinsing actions of flow through the nasal sac are extremely effective. Recent evidence suggests, however, that further attention might profitably be given so-called "natural repellents."

Naturally Occurring Repellents

The experiments of von Uexkull (1895) strongly indicated the existence of at least one naturally occurring shark repellent (in *Aplysia*), even before

this group of chemicals was recognized as an important category of natural products. Hodgson, Mathewson, and Gilbert (1967) reported that holothurin (from Cuvier's gland of the sea cucumber, *Actinopyga agasszi*) elicited avoidance behavior by lemon, nurse, and bonnet sharks tested in the hydrodynamic tunnel. Backus (1973) noted that "... holothurin is a very effective deterrent against predation" and found that fishes seldom even attempt to mouth holothurians, and that holothurians are only rarely eaten by sharks.

Cameron and Endean (1973) and Cameron (1974) have theorized that naturally occurring chemical defenses, repellents, or toxins active against potential predators may be particularly prevalent among the relatively immobile or stationary organisms in marine environments. This is especially true in coral reef ecosystems where great adaptations to stationariness and intensive interspecific predation take place.

An interesting example of a relatively immobile teleost fish that appears to produce a chemical shark repellant was reported by Clark and Chao (1972). *Pardachirus marmoratus*, the "Moses sole" from the Red Sea, secretes a milky substance that, at certain concentrations, is extremely aversive to sharks. It has been demonstrated that sharks will approach *Pardachirus*, but fail to close their jaws on it. Under experimental conditions, the fish's secretion affords protection to other kinds of fish that sharks otherwise eat (Clark 1974).

The source of the secretion in *Pardachirus* appears to be enlarged dermal mucus glands or modifications of muscles controlling movements of dorsal and anal fins. Clark and Chao consider that the toxin is "probably a protein" since it is ineffective after boiling, and it is not affected by freezing. Dr. Eliahu Zlotkin (cited by Clark 1974) found that the exudate has both neurotoxic and hemotoxic effects, and it can be separated into three protein components. A natural inhibitor of the hemotoxin was found, possibly protecting *Pardachirus* from its own poison.

It would be interesting to compare the finding on *P. marmoratus* with the other species of *Pardachirus* inhabiting the littoral waters of the Indian and Pacific Oceans. A closely related genus, *Aseraggodes*, which has no open pores above the base of the dorsal and anal fin rays (Marshall 1964), would also make an interesting comparison.

In addition to the echinoderms cited above, the invertebrate animals offer a potentially rich field for exploration of naturally occurring repellents and toxins. The *Proceedings of Recent International Coral Reef Symposia* (Cameron et al. 1974, Taylor 1977) provide numerous examples of potentially valuable sources of natural repellents and toxins. One example is the highly toxic "palytoxin" produced by the zoanthid *Palythoa*; palytoxin is one of the most highly toxic nonprotein substances known. It is a vasoconstrictor affecting smooth, striated, and cardiac muscle, but repellent properties are as yet untested (Attaway and Ciereszko 1974). Among the arthropods, several highly poisonous species of crabs have been known since the late eighteenth or early nineteenth centuries. Recent studies (Garth and Alcala 1977) indicate that several species produce a heat-stable, water-soluble, and highly toxic substance that quickly affects

nervous systems. The toxic material produces immediate locomotory symptoms, which commonly lead to the death of any animal ingesting them. The role of these poisons in the normal environment of the crabs has yet to be specified, and repellent or toxic actions on normal predators, although suspected, remain to be determined. As with most toxicology studies, the effects of the active molecules on sensory systems, particularly chemoreceptors, are unknown but could prove to be extremely interesting.

The few naturally occurring shark repellents described thus far probably represent only a beginning in the investigation of a promising area. It will be especially interesting to discover whether and how other marine animals may have solved the problems of protecting themselves against shark attack. Realistically, investigations in this area are no more likely than any past studies to identify a repellent capable of incapacitating a frenzied shark. However, it is quite reasonable to anticipate discovery of natural repellents that significantly lower the probability of attack or ingestion by sharks. A combination of basic natural history studies, including the taxonomy, ecology, and behavior of suspected producers of natural repellents and toxins, as well as detailed pharmacological analyses of their secretions, might advance knowledge in particularly interesting and valuable ways at this time.

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CHEMORECEPTION AND ITS INTERACTION WITH FLOW AND LIGHT PERCEPTION IN THE LOCOMOTION AND ORIENTATION OF SOME ELASMOBRANCHS

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INTRODUCTION

There is abundant anecdotal and experimental evidence for the importance
to elasmobranchs of the chemical senses, particularly olfaction. Although
these senses and their biological functions appear fundamentally similar to
those in other fish (Kleerekoper 1969, Hara 1971), we shall review here
some of the more significant literature on the chemosensory biology of
elasmobranchs. No attempt will be made to compile an exhaustive bibli-
ographic survey of the topic. Furthermore, the disparity in available informa-
tion on the two senses restricts the review almost exclusively to olfaction,
revealing a lamentable gap in our knowledge of the role of taste in these
animals.

The scientific, as opposed to anecdotal, information on olfaction in elas-
mobranchs covers three fairly distinct areas: (a) the structure of the olfac-
tory organ and its neural pathways, (b) the role of olfaction in the procure-
ment of food, and (c) mechanisms of orientation through olfaction.

THE OLFACTORY ORGAN AND ITS NEURAL PATHWAYS

The literature on the structure of the organ in fishes in general (Kleerekoper
1969, Parsons 1971, Graziadei 1971, Hara 1971) and in sharks specifically
(Tester 1963) has been reviewed extensively in past years. A few of the main
aspects will be restated here and reference made to some more recent con-
tributions, particularly to new knowledge about the fine structure of the
olfactory epithelium and about the neural pathways.

According to Balfour (1876, 1885), the olfactory organ develops rela-
tively late and becomes apparent later than the eyes and ears (Figure 1).
Immediately in front of the mouth, on the ventral side of the forebrain, a
pair of epiblastic thickenings forms the anlage for the organ, which does not
connect with the neuropore (Berliner 1902). Proliferation of the cells of the
anlage is followed by involution, the formation of a pit, and finally a blind
sac (Figure 2). Numerous folds are formed in the sac by the epithelium,
which is ectodermal in origin.

Although the outer wall of the organ at first consists entirely of sensory
cells, these change progressively into indifferent ectoderm. Cell proliferation
in the remaining sensory epithelium leads to formation of the paired olfac-
tory nerve, which advances toward the brain of the embryo. In *Scyliorhinus*,
the olfactory grooves, at their edges, develop flaplike extensions that project
toward each other and overlap, forming a tube with an incomplete roof and
an anterior and posterior opening (Bütschli 1921, Allis 1919) (Figure 3).
Unlike the condition in teleosts, the posterior opening connects with the
mouth in some species of elasmobranchs. For further detail, the reader is
referred to Bigelow and Schroeder (1948) and Tester (1963), who give an
extensive account of the position and shape of the nostrils in various shark
species.

The olfactory sacs in elasmobranchs are located in the nasal capsules of
the skull. They are divided by several parallel septae, with the narrow spaces
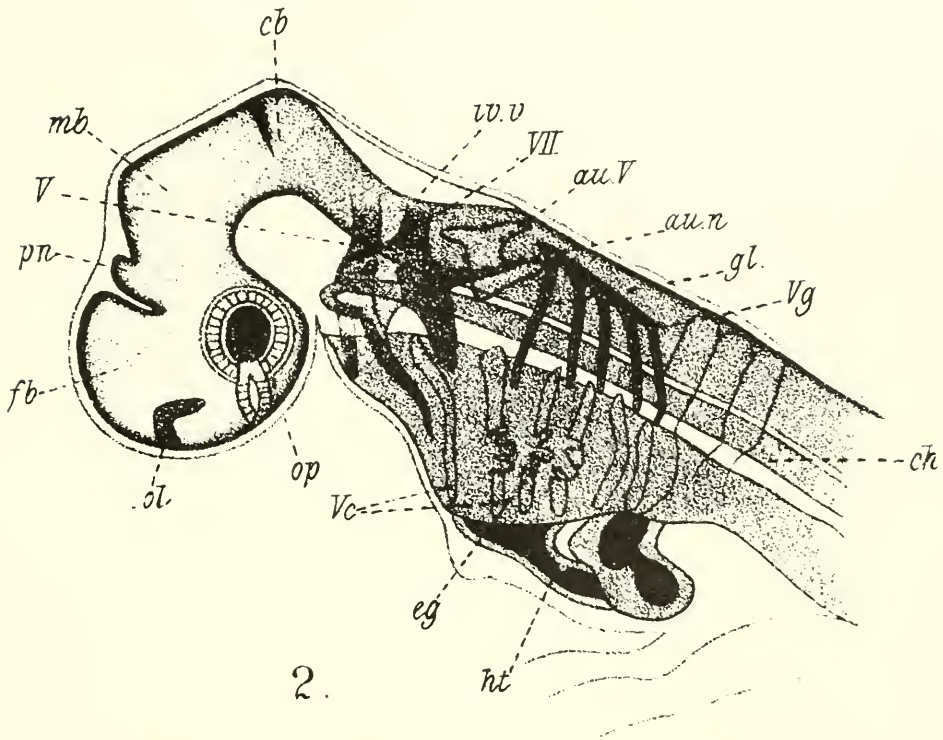
Figure 1 Development of the head in *Scyliorhinus*: eye (op), forebrain (fb), midbrain (md), auditory vesicle (au.v), and olfactory groove (ol). From Balfour (1876).

in between filled with the water to be olfactorially "tested." The olfactory epithelium proper is located mainly at the base of the troughs formed by the septae or lamellae. The somata of the bipolar sensory cells are particularly large in these fish (15 μm across and 20 μm deep). The olfactory sacs of *Mustelus* were described by Sheldon (1909) as a pair of capsules, partly divided by a flap of skin rostrally, and a fleshy ridge caudally. In this manner two incompletely separated external apertures are formed. Like the barbs of a feather, the olfactory lamellae bearing the receptors extend from a median ridge inside each capsule. In addition to the many very thin axons of the bipolar cells, the lamellae receive the endings of the terminal nerve.

For efficient function of the olfactory system the water surrounding the animals' head must be sampled continuously. This is achieved by respiratory movements that draw water into the mouth; part of it enters the more rostral nostril by suction and leaves through the more caudal nostril. Sheldon demonstrated, with the use of a dye, that the water follows the medial ridge in the capsule and is, in part, diverted into the interlamellar spaces. In addition, in a swimming dogfish, water is forced through the nostrils by the displacement of the animal. In fishes generally, similar mechanisms of water
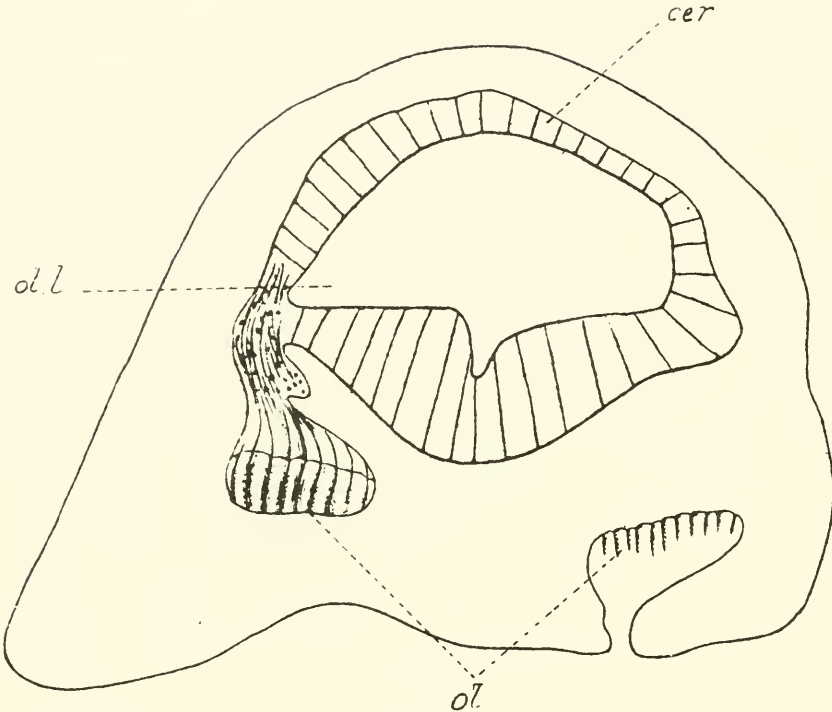
Figure 2   Later development of the head in *Scyliorhinus*: olfactory organ
(ol). From Balfour (1885).

sampling are found, although special accessories for this purpose are fre-
quently encountered (Kleerekoper 1969).

The structure of the sensory epithelium, on the folds or lamellae of the
inner wall of the olfactory sac, is very similar in all vertebrates (Allison
1953). There is no evidence that this epithelium is basically different in
elasmobranchs. In the fully developed olfactory epithelium of fish, three
main elements can be distinguished: olfactory receptor cells and their axons,
supporting cells, and basal cells. Jointly, these elements form a columnar
epithelium arising from a basal structure, the basal lamina, which is separated
from the underlying cartilaginous tissue by the lamina propria.

The olfactory receptor is a bipolar, primary neuron. Its dendritic,
"swollen" extremity, or "olfactory vesicle" (Schultze 1863, Kallius 1905,
Bloom 1954, Vinnikov 1965, Graziadei 1972), directed peripherally, carries,
in most fish, cilia that protrude into the lumen of the olfactory sac. The
number of receptors varies greatly among species (Teichmann 1954). Al-
though the dimensions and shape of the receptor cell and its parts may
differ, the basic structure is similar in all vertebrates. These variations are
found also within species and may reflect maturation of the cells (Andres
1969) which, following degeneration, seem to be replaced by differentiation
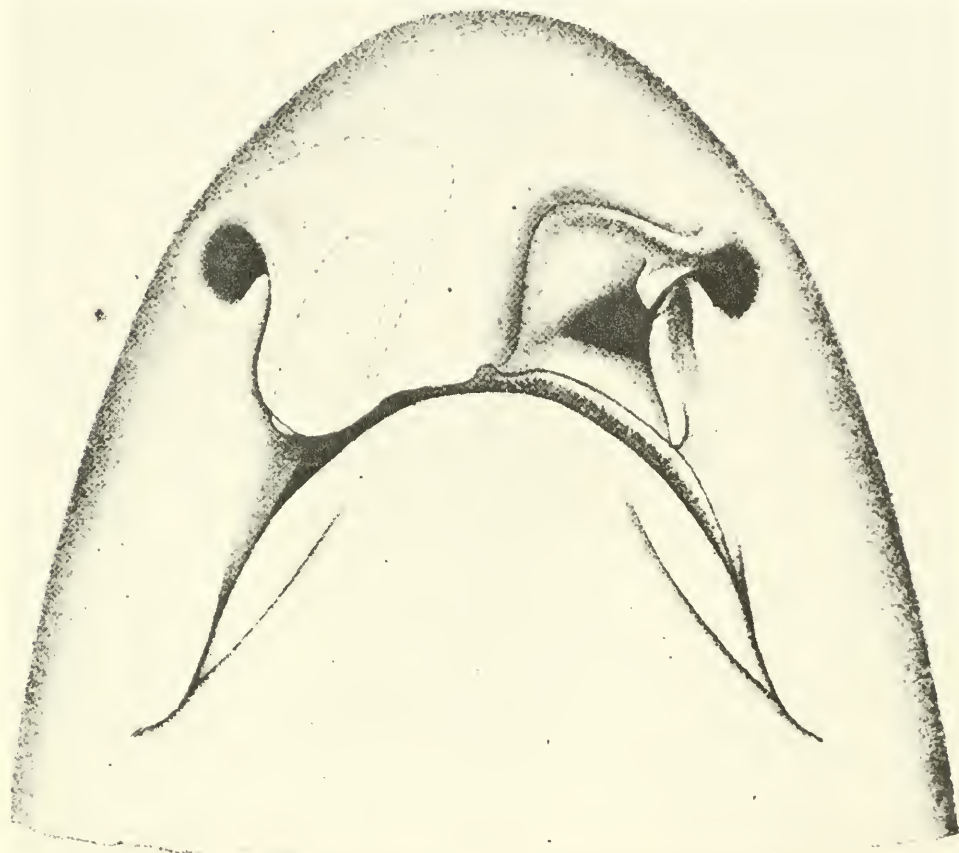
Figure 3 Ventral aspect of the head of *Scyliorhinus*. The nasolabial groove leads from the upper edge of the mouth to the posterior (median) naris. Redrawn from Allis (1919).

of basal cells (Andres 1966, Graziadei and Metcalf 1971). No specific information is available on this process in elasmobranchs.

Although in most species studied the olfactory vesicles bear only cilia, in some species microvilli are present as well, and in some teleosts certain types of olfactory cells carry microvilli but no cilia (Bannister 1965). In *Rhinobatus lentiginosus*, the guitar fish, the olfactory cells have a single apical dendrite ending in an enlarged bulb, protruding above the surface. Although this bulb carries short villi-like protrusions, there are no cilia (Reese and Brightman 1970) (Figures 4, 5).

It has been assumed (Parker 1922) that the cilia, and possibly also the microvilli, carry the receptor sites for chemical stimulation, so that the increased area of exposure created by the presence of cilia and microvilli may be related directly to olfactory sensitivity and acuity. However, removing cilia from the vesicle did not eliminate action potentials in response to odor
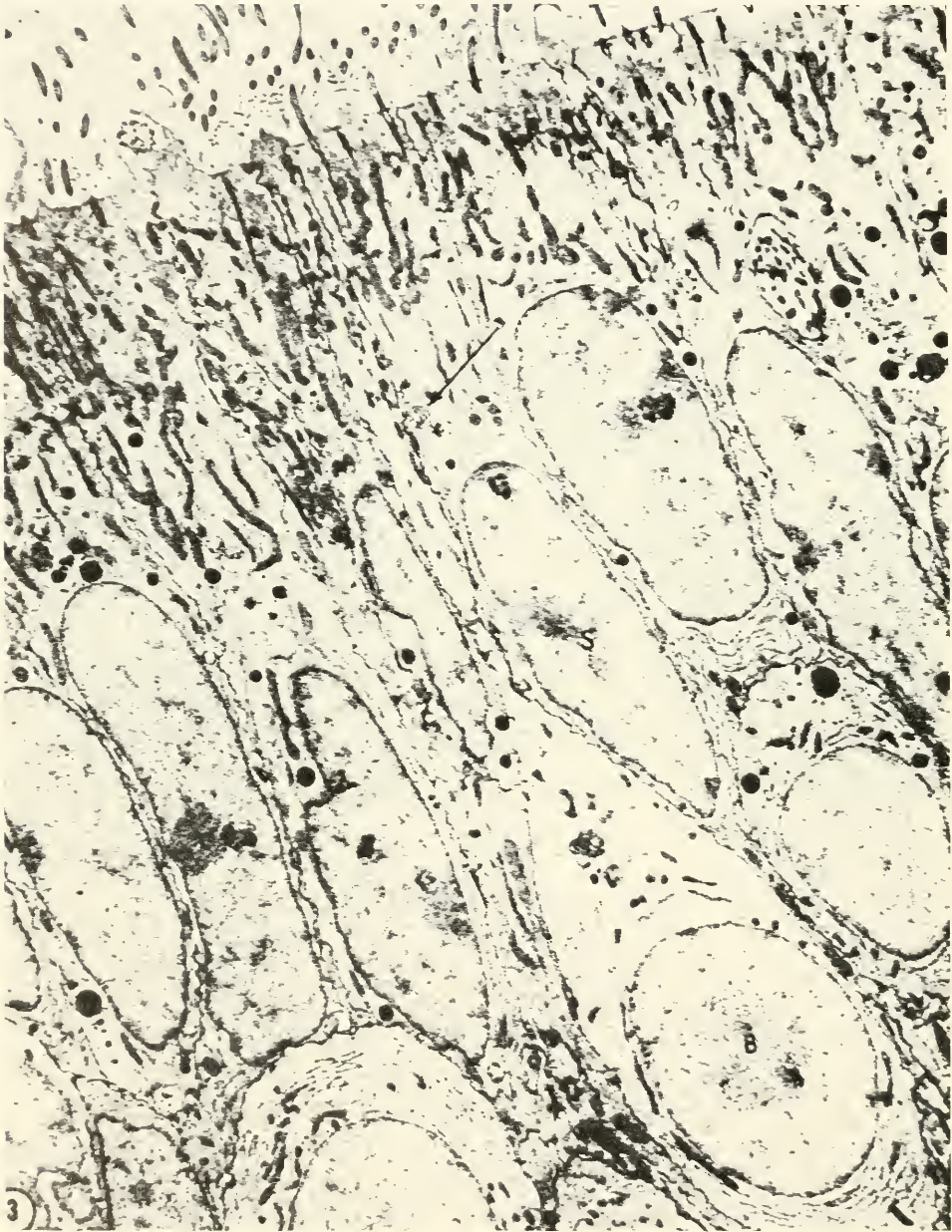
Figure 4 Olfactory epithelium of *Rhinobatus lentiginosus*: round nucleus of olfactory bipolar cell (B), oval nucleus of supporting cell (S). The apical dendrites of bipolar cells in these fishes have a few villous extensions instead of cilia. The surfaces of the supporting cells carry similar villi. These cells also bear motile cilia on their distal ends. From Reese and Brightman (1970).

Figure 5   End of a distal dentrite of an olfactory bipolar cell in *Rhinobatus lentiginosus* These dendrites have small villous processes but no cilia on their tips. From Reese and Brightman (1970).

stimulation in the turtle (Tucker 1967). The finding that the olfactory cells of *Rhinobatus* lack cilia is, therefore, of particular interest, especially if this is common in elasmobranchs, a group that in general has high olfactory sensitivity (Hopkins 1926). Furthermore, there are indications that the whole dendritic extremity of the receptor cell may be engaged in active movement (Vinnikov and Titova 1957, Bronshtein 1963).

Recent observations have confirmed the existence of two or three types of receptor cells in the olfactory epitheliums of fishes (Pyatkina 1974: *Acipenser;* Breipohl et al. 1973: *Carassius auratus*), reported earlier by several authors in a number of vertebrates, including fishes (Dogiel 1886, Morrill 1898, Jagodowski 1901, Le Gros Clark 1956, Neuhaus 1955, Castello 1956, Vinnikov 1956, Bannister 1965, Graziadei 1966, 1971, Andres 1965, 1966, 1968, 1969; Graziadei 1967; Schulte 1972). Although there is no information on the occurrence of different bipolar cells in the epitheliums of elasmobranchs, verification of their presence in other vertebrates, including fishes, is important in that the morphological differentiation may indicate functional differences underlying the mechanisms of odor perception and discrimination. There is the intriguing possibility that different membrane absorption characteristics of the receptor, which could mediate the olfactory stimulus (Wright 1964; Amoore 1952, 1971; Davies 1971; Ottoson 1971; von Herberhold 1969*a*, 1969*b*, 1971, 1972), may be associated with morphologically recognizable differences of the cells (Breipohl et al. 1973). To what extent these morphological differences represent functionally different cell types or merely reflect various stages of degeneration or regeneration of one type has not been clearly established (Bannister 1965). An additional aspect of this problem will be referred to later, in relation to morphological differences in olfactory cell axons.

Below the vesicle, the olfactory cell becomes constricted to various degrees and makes close contact with supporting or sustentacular cells (de Lorenzo 1963, Ottoson 1965) through junctions whose structure has been studied in some detail (Farquhar and Palade 1965, Reese 1965, Reese and Brightman 1970, Robertson et al. 1963). In two elasmobranchs (*Ginglymostoma cirratum* and *Rhinobatus lentiginosus*), Reese and Brightman confirmed the existence of tight junctions with the surrounding supporting cells, just below the cellular apices. Such "tight" junctions, unlike "gap" junctions, constitute a seal that effectively separates the olfactory surface from the underlying structures by forming continuous belts around the bipolar and supporting cells.

Thus, substances in the external medium, carried by the water in the olfactory sac, may be barred from directly mixing with the constituents of the body fluids, and vice versa, unless they diffuse through the tight junctions. The latter possibility has been investigated histochemically by Reese and Brightman (1970). Circulating protein (molecular weight 42 000) was effectively barred from reaching the olfactory surface by the tight junctions, which alone prevented the exchange between subepithelial interstitial fluid and that surface.

Proximally, the olfactory cell body continues into the axon, which joins those of other receptor cells to form bundles that traverse the basal lamina.

The supporting cells surrounding the receptors are high, narrow, and cylindrical. Their distal ends carry microvilli in various numbers and lengths and, in a number of species (including fish), cilia (Langerhans 1876; Watling and Hillemann 1964). In a number of vertebrates these cells actively secrete granules when strong olfactory stimuli are applied (Bloom 1954; Bronshtein and Tvenov 1965; Reese 1965; Frisch 1967; Graziadei 1971). The phenomenon, whose functional significance is unknown, has been described in many vertebrates and may be assumed to occur also in elasmobranchs. It has already been mentioned that adjacent supporting cells are in close contact with each other, particularly near their distal ends, through the array of junctions that form a belt encircling each cell (Graziadei 1971; Reese and Brightman 1970).

Although Bowman's glands, abundant in the olfactory epitheliums of mammals, are absent in fishes, mucus glands have occasionally been described in these animals, including *Mustelus canis* (Asai 1913). In this species, mucus-secreting goblet cells are located in the lamellae. These cells reach the epithelial surface through an extension that compresses the neighboring cylindrical cells (Figure 6).
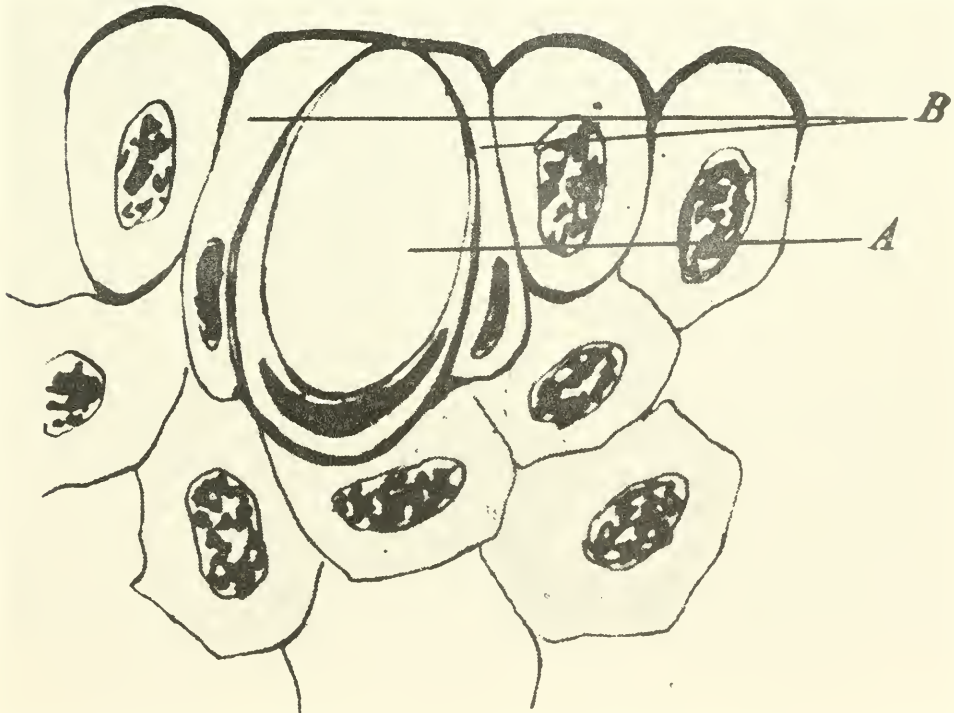
Figure 6 *Mustelus canis* mucus cell (A) in the indifferent epithelium of the free surface of a lamella, in which it compresses the neighboring cylindrical cells (B). From Asai (1913).

Below the basal lamina the axons of the bipolar olfactory cells aggregate in bundles of various size. In fishes, these fila olfactoria join and form fasciculi, which pass through the base of the epithelial folds, or lamellae. Increasingly larger trunks are formed; in some species, these together constitute the olfactory nerve, which is wrapped in the cytoplasm of Schwann's cells and is made up of a great many extremely thin fibers. The nerve passes through the cribriform plate into the cranial cavity. However, in selachians and in a number of other fishes no single olfactory nerve is formed, and the numerous fila continue directly to the olfactory bulb, close to the olfactory sac. The fibers spread over the surface and into the bulb, where they synapse with mitral cells or tufted cells in the olfactory glomeruli.

The basic structure of the olfactory bulb is similar in all vertebrates from cyclostomes to primates. An early study of this structure in *Mustelus* was made by Asai (1913). According to this author, the glomeruli in this species measure 0.455-0.050 mm in size and can be irregularly distributed in the mitral layer. In some areas they are arranged in two or three rows. Andres (1970) distinguishes five histological layers: (a) olfactory nerve layer, (b) glomerular layer, (c) mitral cell layer, (d) plexiform layer, and (e) periventricular layer (Figure 7). (In terrestrial vertebrates an additional internal plexiform layer can be defined.) He does not recognize the "granule cell layer," frequently referred to in the literature. In *Scyliorhinus canicula* the mitral cells have particularly thick dendrites (Figures 8, 9) and the plexiform layer is very rich in granule cells.
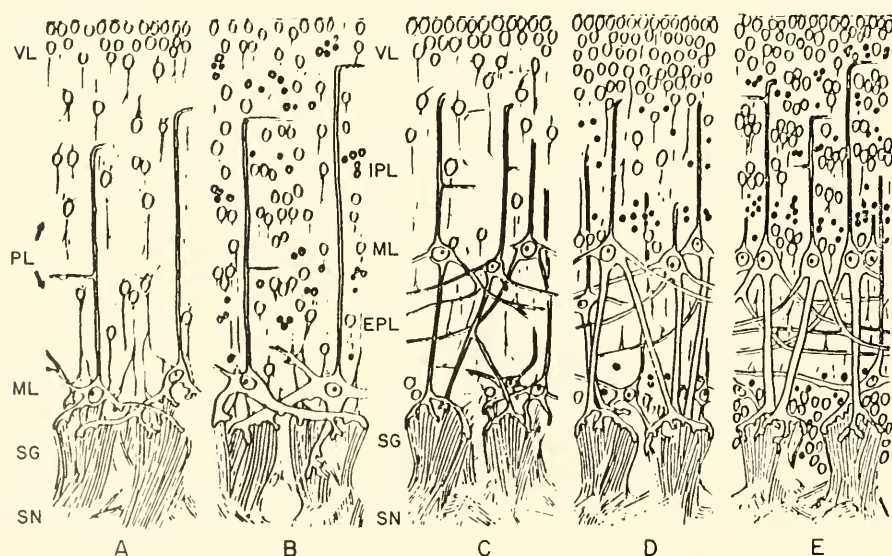
Figure 7 Fibers and cytoarchitecture of vertebrate olfactory bulbs: olfactory nerve layer (SN), glomerular layer (SG), mitral cell layer (M), plexiform layer (PL), periventricular layer (VL), and external plexiform layer (IPL). (a) Lamprey, (b) elasmobranch, (c) amphibian, (d) reptile, (e) mammal. From Andres (1970).
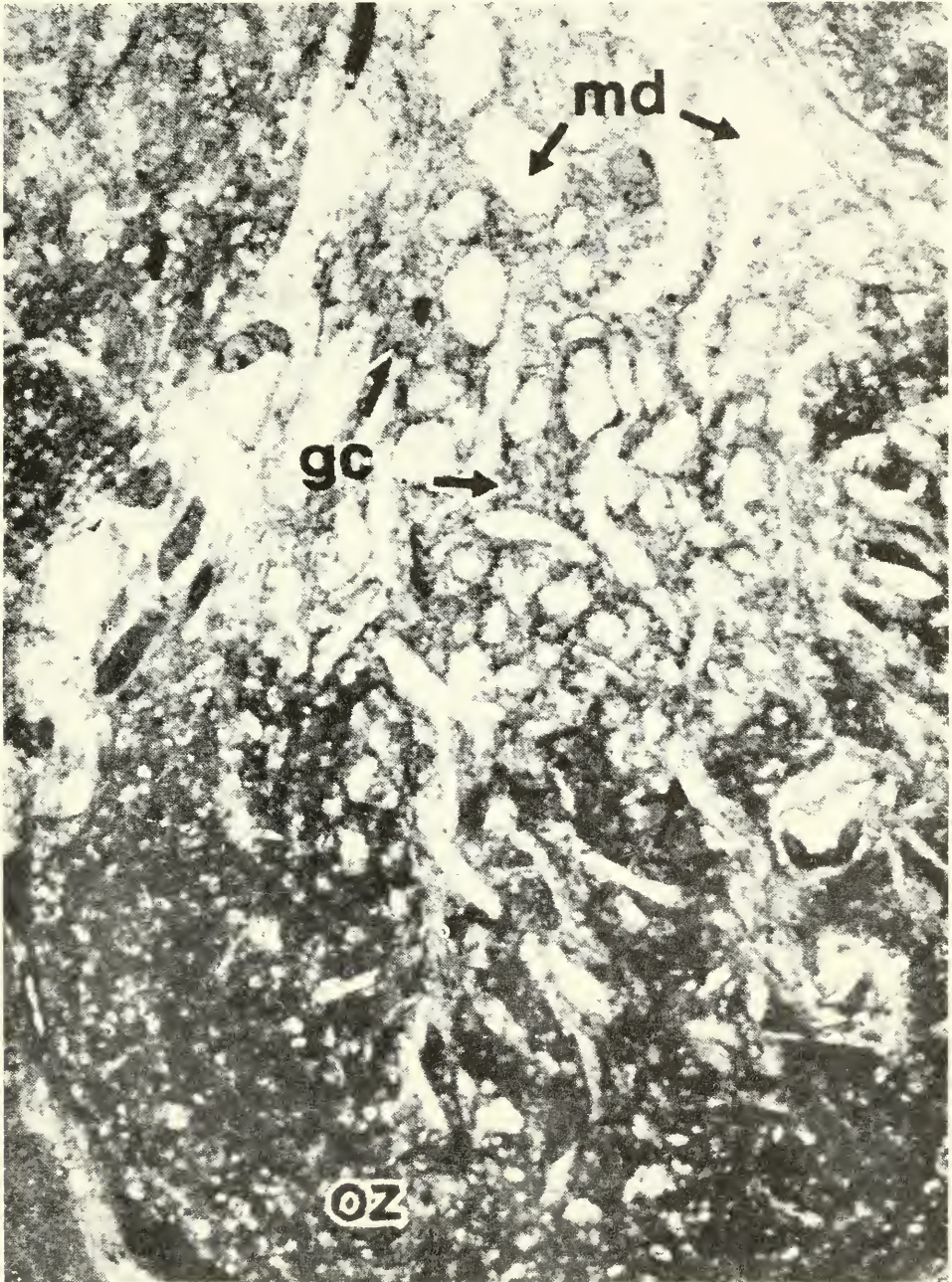
Figure 8 *Scyliorhinus canicula* olfactory bulb: mitral cell dendrites (md) mixed with granule cell axons (gc) in inner region. From Andres (1970).
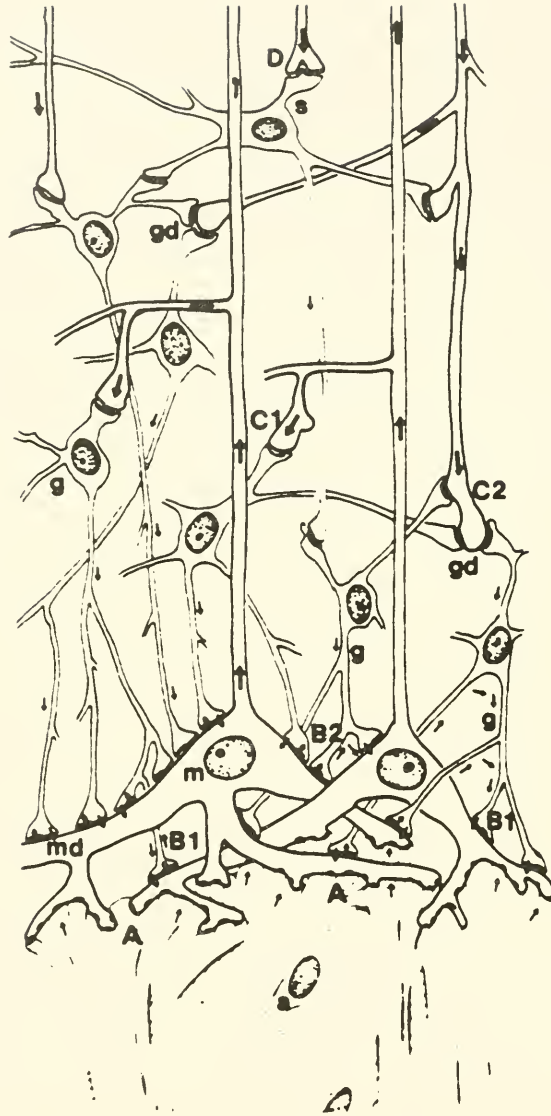
Figure 9 Synaptic connections in the olfactory bulb: mitral cell (m), granule cell (g), and fila olfactoria (A). From Andres (1970).

In contrast with those of mammals, each mitral cell usually is connected with several glomeruli (Figure 10). In turn, granule cells make contact with mitral or tufted cells through synapses of various types, depending on whether they are axodendritic, axosomatic, dendroaxonic or somatoaxonic. The latter two are of the so-called reciprocal type, found on the dendrites and somata of the mitral cells of fish. These synapses are part of a most
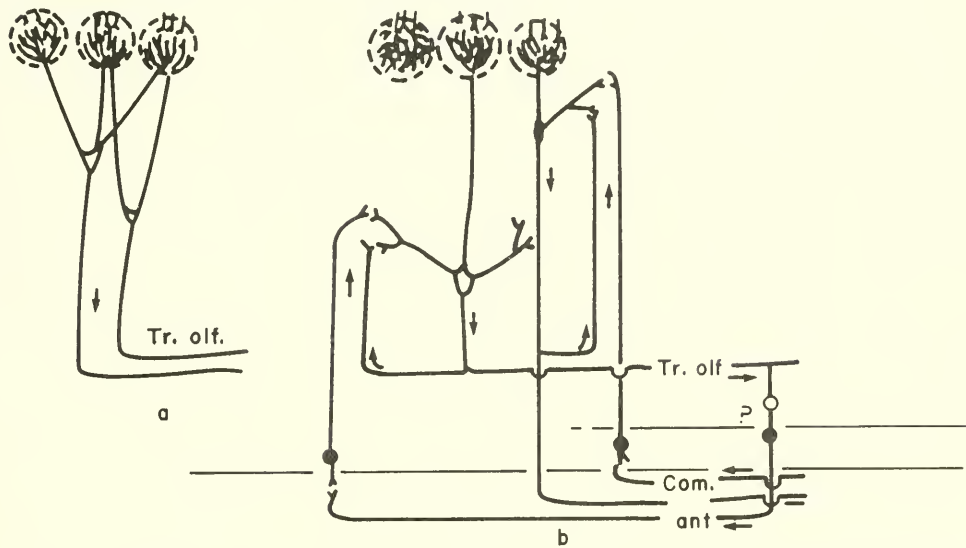
Figure 10   Glomerular connections in (a) fishes and (b) mammals. From Allison (1953).

important inhibitory system, which makes possible suppression of peripheral information carried by the fila olfactoria when it arrives at the bulb. The reciprocal, or two-way, synapses can either mediate the inhibition directly on arrival of the impulses or they can be activated by granule cells when these are stimulated via recurrent collaterals of mitral cells (Nicoll 1971; Price 1968; Westecker 1970; Yamamoto 1961; Yamamoto and Yamamoto 1962). Synaptic membrane complexes on dendritic terminals of the granule cells, mentioned by Andres, are probably related with those inhibitory functions.

The fact that the olfactory cell axons contain varying numbers of microtubules (2 to 18) again suggests functional differences between olfactory receptors; this may be related to different odor discriminatory properties of these cells.

The great similarity in cytoarchitecture of the olfactory bulb in all vertebrates is paralleled in its electrophysiological characteristics. Orthodromic stimulation produces in the bulb an electrical response that has three parts (Bruckmoser 1973): the impulse from the olfactory fila, the response of the mitral cells, and inhibitory activity by the granule cells.

There is general agreement that much of the processing of olfactory information occurs in the bulbs. In the rabbit, for example, the glomeruli seem to behave as individual discriminators; specific odors may stimulate only some glomeruli (Leveteau and MacLeod 1969). Much less is known about such functional detail in fish, but the evidence indicates that the more caudal telencephalon receives from the bulb highly processed information, mainly through the axons of the mitral cells, bundled into the lateral olfactory tract. The telencephalic region in *Scyliorhinus* which receives this information is

represented in Figure 11. The lateral ventricles form hollow spaces whose dorsal walls constitute the pallium.

Until recently the available evidence indicated that the lateral olfactory tract in sharks carries the extensively processed olfactory information to most regions of the telencephalon, which therefore seemed to be mainly concerned with olfactory and gustatory function (the "olfactory brain": Edinger 1980; Ariëns Kappers 1909; Ariëns Kappers et al. 1936; Nieuwenhuys 1967). This interpretation of old standing had to be drastically reviewed as a result of recent findings which demonstrated that the olfactory tract projects onto only a small zone of the lateral wall of the telencephalon in the nurse shark (Figure 12) (Ebbesson and Heimer 1968, 1970; Veselkin and Kovacevic 1973), whereas higher order olfactory areas do not seem to have extensive projections to the hemispheres either (Ebbesson 1972).

This raised phylogenetically important question of what the functions of those large telencephalic regions that apparently do not receive olfactory information could be (Schroeder and Ebbesson 1974). Earlier (Ebbesson and Schroeder 1971) it was shown that the telencephalon in the nurse shark receives optic afferent nerves; this suggests that the forebrain may have become a sensory integration center early in phylogeny. Bruckmoser (1973) has suggested that such an integrative function may play a role in migratory and homing behavior in lower vertebrates. Furthermore, Ebbesson and his collaborators demonstrated that the retina (Ebbesson and Ramsey 1968), cerebellum (Ebbesson and Campbell 1973), spinal cord, and tectum (Ebbesson et al. 1972) send axons to the dorsal thalamus, which may therefore function as a relay for sensory information to the nonolfactory telencephalic areas. The pathways of some of the nonolfactory afferents were recently
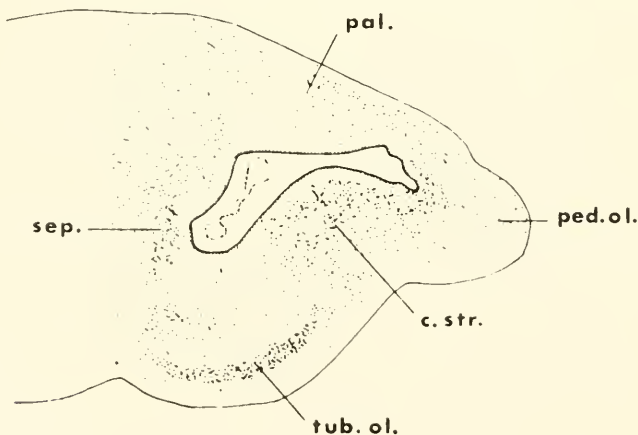
Figure 11 Transverse hemisection through the telencephalon of *Scyliorhinus*: pallium (pal), pedunculus olfactorius (ped. ol.), corpus striatum (c. str.), tuberculum olfactorium (tub. ol.) and septum (sep.). From Nieuwenhuys (1967).
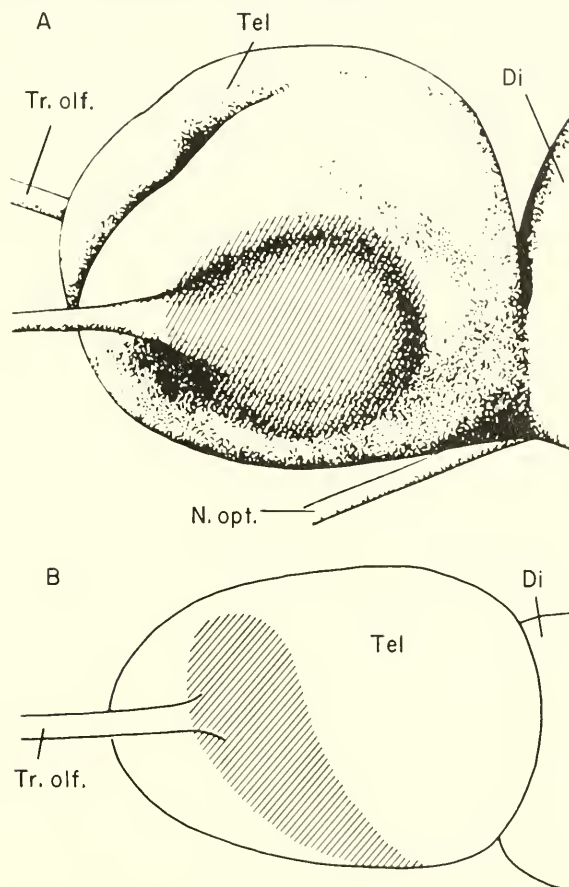
Figure 12 (A) *Torpedo ocellata* and (B) *Gingly-mostoma cirratum* telencephons. Shaded areas are projection areas of the lateral olfactory tract in the lateral olfactory region of the telencephalon. Lateral views. From Bruckmoser (1972).

described (Schroeder and Ebbesson 1971, 1974). Both electrophysiological (Cohen et al. 1973; Veselkin and Kovacevic 1973) and behavioral (Graeber and Ebbesson 1972; Graeber et al. 1973) evidence has been presented for visual functions of the telencephalon, independent of the optic tectum.

Observations on evoked potentials in primary and secondary olfactory projection areas in elasmobranchs by Bruckmoser and Dieringer (1973) confirmed that the secondary olfactory area is much more restricted than previously believed. Electrical stimulation of the olfactory epithelium and the bulb in *Scyliorhinus* evokes secondary olfactory potentials in the forebrain with conduction velocities of 1 to 2 m/s in the fibers of the olfactory tract.

Gilbert et al. (1964) described EEG patterns of the olfactory lobe associated with olfactory stimuli in unanesthetized lemon sharks (*Negaprion*

brevirostris), bonnet sharks (*Sphyrna tibro*), and nurse sharks (*Gingly-mostoma cirratum*). Surface potentials of the telencephalon increased in frequency and amplitude during chemical stimulation with various substances, such as the body fluids of crabs and glycine. Negative potentials of various amplitudes, followed by lower potentials of opposite polarity, were recorded 5 mm below the surface in the anterior halves of the lobes of the forebrain, in response to extracts of tuna meat, dl-forms of glutamic acid, glycine, cystine, serine, tuna blood, body fluids of lobsters and crabs, and amine F, since identified as isoleucine methylester, an olfactory attractant for lampreys, some teleosts, and sharks (Kleerekoper 1963 and unpublished data). In later work, Hodgson et al. (1967), using similar techniques to record EEG patterns in free-swimming sharks, restrained in a 60-cm-by-420-cm tank, studied EEG characteristics as a function of olfactory stimulation with a variety of substances and behavioral responses. Close correlation between forebrain and medulla EEG patterns, and the behavior of free-swimming sharks was established in response to various electrolytes, amines, and amino acids. In nurse sharks, these responses were not affected by removal of both barbels, but experimentally induced anosmia abolished both the EEG and behavioral effects of chemical stimulation.

THE ROLE OF OLFACTION IN FOOD PROCUREMENT

It is of historical interest that some of the first scientific studies on olfaction in fish included observations on food procurement in elasmobranchs (*Scylio-rhinus*). Although Fabricius reported as early as 1753 on the response of sharks to the scent of fouling meat, the first experimental investigation of the sense of smell in elasmobranchs was done by Bateson (1890) in a study on olfaction in fishes in general. By observing the behavior of normal and anosmic animals he concluded that a number of fish species, including *Scyliorhinus caniculus*, *Raja cattis*, *Squatina squatina*, and *Torpedo*, located their food by olfaction. The normal fish, exposed to food odors in an experimental tank, began to respond to the scent after it "had been diffused through the water." They "swim vaguely about and appear to seek [the food] by examining the whole area pervaded by the scent, having seemingly no sense of direction whence it proceeds." The "process of search is equally indirect and tentative by day and by night, whether food is exposed or hidden in an opaque vessel, whether a piece of actual food is in the water or the juice only, squeezed through a cloth and, lastly, whether the fish be blind or not." Of particular interest is the observation that the response never lasted longer than 15 min after introduction of the odor source into the experimental tank. Evidence will be presented later that *Ginglymostoma* continues to respond to food odor, presented in similar conditions, for long periods.

The choice of the species or even group of fishes by von Uexküll (1895) was incidental to the purpose of his study, which was to decide whether olfaction could function in the aquatic medium and to gather experimental

support in a theoretical and philosophical polemic with the chemosensory expert of the period, W. Nagel. The latter had maintained, in a lengthy treatise (Nagel 1894) that chemical perception in water could be mediated only through taste and the "general chemical sense," a viewpoint stated earlier by Duméril (1807a, b) and Weber (1847) but shown to be incorrect by Aronsohn (1886). In passing, it should be mentioned here that the problem was studied by several workers over the next 25 years; the question was definitely settled when Matthes (1924a, b) demonstrated that in *Triton* olfaction functions equally well in the terrestrial and aquatic stages of the life history of this animal.

Von Uexküll had observed earlier that starved sharks display high olfactory sensitivity. He deprived six animals of food for several weeks after removing, by scraping, the olfactory epithelial surface in two of the subjects. These anosmic animals remained motionless and never reacted to food (dead but fresh fish) placed in front of their nostrils. The normal shark, on the other hand, started search movements with 3 to 6 min. They also became greatly excited when von Uexküll, after manipulating food fish, rinsed his hands in the aquarium.

Von Uexküll attempted, probably for the first time in sharks, to experimentally distinguish gustation from olfaction. For example, he observed that mashed fishbait mixed with quinine was as readily located by the shark as normal bait, was taken into the mouth, but then spit out. The bait was ingested only after it had remained in the water long enough for the quinine to be washed out. The author concluded that the quinine stimulated the chemical sense of the mouth but not that of the olfactory surface and that both taste and olfaction were involved in the observed behavior. He considered, however, that bitter food was not a normal stimulus situation and that in natural conditions a nutrient item would be localized by an animal, while an inedible one would not. For example, the apparently noxious marine slug, *Aplysia*, is normally ignored by these shark, but when active search was excited by placing food fish near such a snail, several sharks captured it but readily spat it out again.

Von Uexküll interpreted these results as confirming the lack of olfactory stimulation by snail substance and attributed the "erroneous" capture to poor visual discrimination between the food fish and the snails. It is of interest that the indiscriminate capture and even ingestion of nonfood items by sharks has often been reported. Additional observations by the same author refer to the effects of currents on the search movements of the animals and to the occurrence of spiral pathways in the course of searching. Although descriptive, these observations are of particular interest in connection with recent analyses in this laboratory of such search movements, which will be referred to later.

Frequently quoted observations, all made within a period of a few years (Parker and Sheldon 1913; Sheldon 1909, 1911; and Parker 1909, 1912, 1914), essentially confirmed Bateson's and von Uexküll's findings and established the significance of olfaction in recognition and procurement of food by sharks. Sheldon (1911) demonstrated that *Mustelus* will locate and attack

food (crab) readily, provided that body juices from the bait diffuse into the water, even when no visual cues are available as to the presence of the food. A piece of cheesecloth soaked in crab body juice and attached to a stone was repeatedly attacked. The animals approached all odorous preparations by performing "searching" swim patterns, followed by circling movements around the odor source. Animals that had been made anosmic by plugging the nostrils with cotton wool ignored crabs placed in the experimental tank. Removing the plugs restored the normal behavior pattern. Similar results were obtained by Sheldon when nerve transection had produced anosmia.

Further work by Parker and Sheldon (1913) did not add significantly to the basic understanding of the problem. *Mustelus canis* was observed to locate injured crabs more quickly than live ones. The presence in the tank of crabs with flesh exposed elicited in nearby sharks rapid locomotor responses, such as quick turning, and search movements over the bottom. "The head was moved rapidly from side to side as the fish swam slowly, coursing, in gradually diminishing circles, 2 or 3 inches from the bottom." When the shark came within 5 to 8 cm, it seized the crab suddenly, shaking it violently from side to side. Vision did not appear to play a role in the localization. Sharks were often observed biting into the bottom of the tank at the exact site where a crab had lain. These and earlier observations confirmed that these sharks recognize and localize food through some chemical sense. Subsequent experimentaion with anosmic animals confirmed the essential role of olfaction in that behavior.

MECHANISMS OF ORIENTATION THROUGH OLFACTION

The important question of the mechanism of orientation through olfaction was raised by Parker (1914), also in *Mustelus*. If odor in the water exerts "a directive influence," he reasoned, then this influence should be affected if the shark were rendered anosmic unilaterally. The direction of the movements of normal and unilaterally anosmic animals, as well as the time required to locate the odor source of crab meat, were recorded. The time was about the same in the two groups of fish, and the normal subjects made an equal number of left and right turns. The experimental animals, which had one nostril obstructed with a cotton plug, turned predominantly toward the side of the functional nostril, making typical circus movements like those described earlier in invertebrates with unilateral sensory dysfunction. Removing the plug reestablished the normal locomotor pattern, consisting of an equal number of left and right turns. Parker held these results to demonstrate that the shark, in approaching the source of an odor, turns consistently in the direction of the nostril that receives the strongest olfactory stimulation, localizing the food, therefore, by osmotropotaxis. In a unilaterally anosmic animal the movements must become predominantly circular in pattern, although in sharks, unlike invertebrates, a number of movements continue to be made in the direction of the nonfunctional nostril. This results in figure-eight locomotor patterns. The noncircus movements

were categorized by Parker as random movements, so that food localization would be mediated by a combination of osmotropotactic and random movements.

Nearly half a century passed before a further investigation was made of the role of olfaction in the recognition of and orientation toward a food source, by Teichmann and Teichmann (1959) in *Scyliorhinus canicula, S. stellare,* and *Mustelus laevis.* The experimental approach differed from that in earlier work in that attempts were made to condition the animals to olfactory cues from synthetic odorants, mostly β-phenylethyl alcohol. Training was done by first placing odorant-impregnated material (cellulose) and then a piece of food (fish flesh) near the head of an immobile fish. Eventually, one animal learned to associate the synthetic odor with the food and responded to the conditioned stimulus alone, first with general excitement and then with specific search movements. The authors observed that the duration of the first phase of the response (general excitement, increased respiration, fin movements) became greatly reduced in the companions of an animal that had already become excited and had initiated search movements. The response consisted, therefore, of alarm and orientation phases, but unilateral anosmia affected neither these responses nor the turning behavior of *S. canicula.* Unlike the other two species, *Mustelus laevis,* excited by the odor, often oriented almost directly to the source once general localization had been achieved. Exact localization, by means of sharp turns, followed. It is of interest that this species responded to unilateral anosmia in exactly the same manner described by Parker (1914) for *Mustelus canis,* i.e., with search movements in the direction of the functional nostril.

Tester (1963*a, b*) made extensive observations on responses to natural and artificial odors in juveniles and adults of various species of sharks (*Carcharinus melanopterus, C. menissorah, Sphyrna lewini,* and *Galeocerdo cuvier*). In addition to general locomotion, behavior patterns such as sudden turns, circling, and head-shaking were used as criteria for the behavioral response to the chemical stimuli, which were introduced below the water surface through tubes.

Based on the activity of the animals during a series of 3-min test periods, with and without olfactory stimulus, numerous responses were defined (no response, sensing only, weak attraction, strong attraction, weak repulsion, strong repulsion, alarm reaction, etc.). Such classification was difficult to interpret because of variability in the responses, and the problem of establishing a reliable bioassay was thereby recognized. In general, the extracts of food substances were attractive to the shark. Even here there was variability in response, making it difficult to compare the attractiveness of the various materials used (flesh of various fish species and invertebrates). Fresh human blood also was "attractive," at concentrations estimated to be at 0.1 to 0.01 ppm of seawater, and produced various locomotor responses. Blinded blacktip sharks (*Carcharinus melanopterus*) were attracted to live fish of various species, presumably by their body odor, as well as to water in which fish had been kept. The response was greatest at the onset of stimulation but then quickly decreased, a result which Tester attributed to habituation. However,

a renewed "hunting" response could be elicited when the odor from "fright-ened" or "excited" fish was introduced into the sharks' environment. The author ascribed this renewal of response to stimulation by a "new odor," released by fish under stress but not by quiescent individuals.

Injury of the fish's skin was not necessary for the release of the odor in question, the source of which, therefore, was not likely to be found in the "body juices." Tester raised the question of whether the attractant, ap-parently emanating from fish under stress, might be chemically related to "Schreckstoff," released through the injured skin of minnows and other cyprinids (von Frisch 1941), and identified as a pterinlike substance by Hüttel (1941), Pfeiffer and Lemke (1973), and Pfeiffer (1975). Hobson (1963) confirmed the attractiveness to sharks in Eniwetok lagoon of water in which uninjured but "agitated" groupers had been kept. A siphon releasing that water was located from a distance by whitetip (*Triaenodon obesus*) and grey (*Carcharhinus menisorrah*) sharks which followed the odor "trail" up-stream. However, neither Tester nor Hobson has demonstrated the release by fish under stress of a specific odor substance that would allow sharks to distinguish such individuals from "relaxed" fish. Furthermore, it would seem difficult to attribute an adaptive advantage to the ability to discriminate between quiescent prey and prey under stress. Nevertheless, the problem is intriguing and deserves a careful experimental analysis of behavior in re-sponse to body odor from quiescent prey and that under stress.

Hobson (1963), in a general study of the feeding behavior of the grey shark (*Carcharhinus menisorrah*), blacktip shark (*C. melanopterus*), and whitetip shark (*Triaenodon obesus*), dedicated several field experiments to the role of olfaction in that behavior. In assessing that role, the criterion chosen was the relative time required to locate an uninjured fish, struggling from a line, from downstream or upstream directions. The rationale was that, if the directional cues were olfactory, approach from the downstream direction should predominate and be faster than that from upstream and random directions. In 9 out of 10 trials, involving 16 grey and 2 whitetip sharks, the approaches were indeed from the downstream direction. As Parker had done before him, Hobson raised the important question of "whether sharks can follow an olfactory cue directly to its source in the absence of other cues." Based on Parker's (1914), Tester's (1963), and his own results, Hobson (p. 179) postulated that sharks are "capable of follow-ing an olfactory trail in running water, particularly when the current is strong and the trail narrow, thus forming what would essentially be an olfactory corridor." He also concluded that olfactory stimuli release explora-tory behavior in grey sharks but that feeding requires an additional stimulus, such as vision.

Hodgson and Mathewson (1971) (and Mathewson and Hodgson 1972) further pursued the analysis of orientation behavior in response to chemical stimulation in lemon sharks (*Negaprion brevirostris*) and nurse sharks (*Gin-glymostoma cirratum*). They reexamined the long-standing question of whether "true gradient searching" is used to orient toward the highest con-centration of odor substances or whether these stimuli only trigger anemo- or

rheotaxis that would lead the organisms upstream and therefore to the source of stimulation. They photographically recorded locomotor tracks made by sharks kept in pens in a shallow lagoon near the Bahamian island of Bimini, for periods of up to 15 min, as the animals responded to dissolved chemical stimuli delivered through tubing below the water surface. Because the water currents differed in various areas of the pens, the locomotor patterns could be studied as a function of the direction and rate of water flow.

When the chemical reached a resting nurse shark, the animal responded with to-and-fro movements of the head and approached the stimulus source along an S-shaped track. The authors concluded that this species locates the source of the stimulus through true "gradient-searching" behavior. The responses of the lemon shark did not allow for as clear an interpretation of the orientation mechanism involved. However, the authors felt that in this species chemical stimulation merely triggers upstream orientation in the strongest current, which, depending on the experimental situation, does not necessarily lead the animal to the stimulus source. The authors pointed out that "the use of chemical stimuli primarily to trigger a rheotaxis, as in the lemon shark, makes for simpler demands upon the chemosensory receptor than does any kind of gradient-searching mechanism."

The rheotaxis-release mechanism had been proposed earlier by Kleerekoper (1969), based on a laboratory analysis of locomotor behavior in *Mustelus, Scyliorhinus*, and some teleosts (see below) and is similar to that described for snails (Copeland 1918; Henschel 1932), insects (Flügge 1934; Steiner 1953; Murr-Danielczick 1930; Otto 1951), *Planaria* (Doflein 1926) and *Triton* (Czeloth 1931), which respond either anemotactically or rheotactically to attractant odors. Dijkgraaf (1975) recently reported that *Scyliorhinus canicula* responds to the odor of dead prey with alarm behavior consisting of searching movements, the direction of which seemed determined, in part, by the stimulus concentration. Although localization may be rapid, snapping at the food source seemed dependent on tactile stimulation and, possibly, electrolocation through the ampullae of Lorenzini, but not on vision. Localization of living prey may be enhanced, at least at short distances, by perception of water movement by means of the lateral-line system. In one-directional water flow, rheotaxis may contribute to quick orientation toward the odor source.

STUDIES OF LOCOMOTOR BEHAVIOR AND ORIENTATION MECHANISMS IN THE AUTHOR'S LABORATORY

Even a summary review of the evidence on which analyses of orientation mechanisms in fishes have rested reveals that a conspicuous characteristic of general locomotor behavior in these (and probably most other) animals has been neither recognized nor accounted for. This characteristic is variability over time in an individual and among individuals of a species. The difficulty that such variability creates in attempts to characterize and quantify locomotor responses to various experimental situations, though obvious, has

been ignored by most workers. The reasons for this are equally obvious; accounting for variability requires that it be statistically defined, which in turn requires quantitative description of locomotor behavior over time, a task of considerable experimental and theoretical complexity. However, an experimental, quantitative assessment of oriented as well as general locomotor responses to chemical and/or physical cues in the environment cannot be rationally attempted before spontaneous locomotor behavior and its variability over time, in the absence of such cues, is understood, at least to a certain degree. It is mainly this consideration that gives the rationale for the experimental approaches to the study of orientation in fishes in this laboratory.

It was considered that, ideally, the definition of the general locomotor behavior of a species should take the form of a model so that changes in that behavior, resulting from response(s) to sensory cues, might be detected in modifications of that model and, therefore, subject to quantification.

It became necessary to define those characteristics whose values might adequately describe locomotor behavior over time and, therefore, should be incorporated in the model. A locomotor pattern is the consequence of temporal relationships between the magnitudes (direction) and frequencies of turns, the lengths and orientations of the straight pathways or "steps" between these turns, and the velocities of the fish. Experimentally, therefore, the problem was to devise ways to monitor the above variables in free-swimming fish. Cinematography, either continuous or intermittent, quickly proved inadequate for the purpose because variability of the values monitored and the need to define that variability almost always required monitoring of the movements over extended periods. Furthermore, visual measurement of large numbers of angles, steps, and time lapses proved to be impracticable. Nevertheless, some very useful data were gathered cinematographically; they lead to the recognition of logarithmic spiral configurations in the swimming patterns of *Ginglymostoma* (see below).

Consequently, through various stages of improvement, two monitor systems were developed (Kleerekoper 1967, 1969; Kleerekoper et al. 1969). An updated description was prepared recently (Kleerekoper 1977) and is here summarized. The two systems have different but complementary capabilities and are frequently used in conjunction. The first system (monitor I) has three versions in operation in this laboratory. One is for larger fish, such as juvenile sharks up to about 80 or 90 cm long, and two are for smaller fish; the latter monitors, in addition, are equipped for orientation studies involving polarized light.

Only the shark monitor will be described here. It consists of a cylindrical steel tank, 549 cm in diameter and 103 cm deep (Figure 13). Sixteen radially oriented, hollow boxes (A), 97 cm high and 138 cm long, partially divide the tank into 16 peripheral compartments, which communicate centrally with the remaining open space (241 cm) of the tank. Near their central, free extremities, the lateral walls of the boxes have narrow vertical windows. In alternate boxes, alined behind these windows, are white tubular fluorescent lamps (40W), covered with red filters. Behind the windows of the remaining
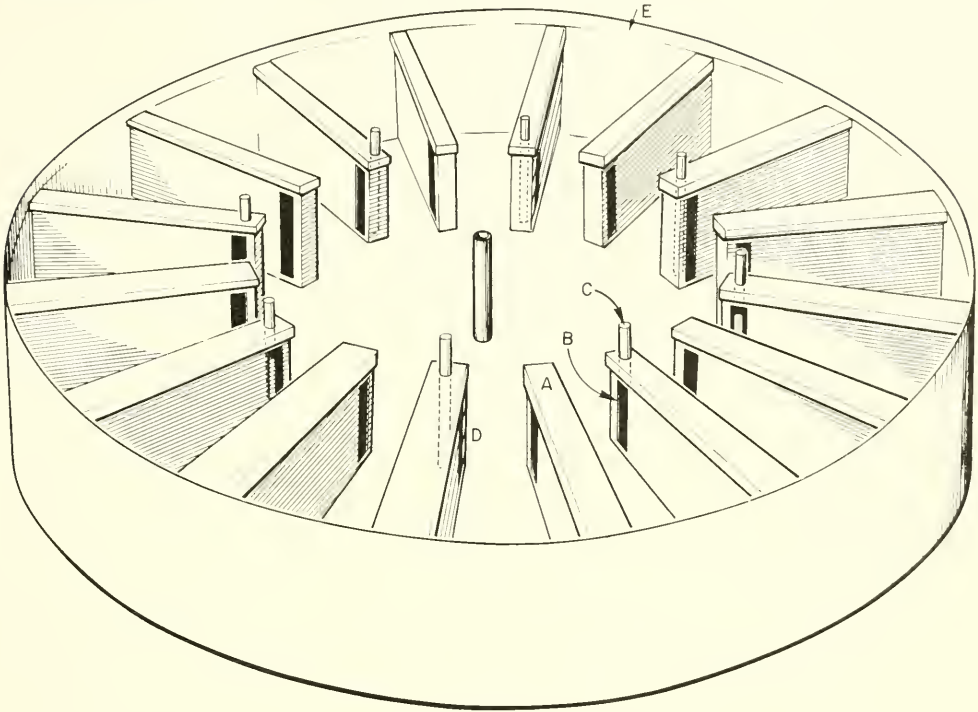
Figure 13 Shark monitoring tank: radially oriented hollow dividers (A); vertical windows (B), behind which are either fluorescent lamps and red filters (C) or banks of photoconductive cells (D); peripheral channel (E) through which water is supplied to the individual compartments.

boxes are mounted banks of eight photoconductive cells, uniformly spread with their photosensitive surfaces directed toward the windows of the neighboring boxes containing the light sources. Stops in front of the photocells reduce light scatter and thereby heighten the responsiveness of each cell to reductions in light intensity, resulting from the passage of a fish at the level of that cell. Thus, in the tank as a whole, 16 photoelectric gates are formed, passage of the fish through which is recorded, with the time, on paper tape by an electronic recorder. The record, therefore, reveals the time of entry and exit and the number of the compartment in question. It is afterwards transferred to a computer disk pack, whence it can be retrieved for analysis and computation of various locomotor characteristics. Frequency distribution of entries, time spent in each compartment, orientation angle on leaving a compartment, sequence of pathways as a function of time, and velocities are examples of the information computed from the raw data.

The main, cylindrical body of the monitor tank is surrounded by a continuous channel (B in Figure 13) of the same depth as the tank. Synthetic seawater, treated by biological sand and diatomaceous earth filters, enters channel B, where it is maintained at constant high level by a motorized valve controlled by a water level sensor. The water in the channel then enters each

compartment through a subsurface orifice and flow-control valve, traverses the compartment and the central open area, and leaves through a standing pipe in the center of the monitor tank. The rate of flow can be regulated so that the same or different flow rates can be established in the compartment. The monitor is surrounded by a light trap and is located in a dimly lighted laboratory room. Diffused overhead lighting is available for some experiments. Chemical or control solutions can be introduced into one or more compartments by means of solenoid pumps, the stroke paths of which can be electronically controlled as to length and frequency. Sound or light sources can be added, singly or in combination with other stimulus modalities.

The second system (monitor II) is composed of a tank, 5.0 m × 5.0 m × 0.6 m (Figures 14, 15), in the floor of which is embedded a square matrix of 1936 photocells on 10-cm centers, photosensitive faces upward. A continuous field of collimated light, suspended over the tank activates the photocell matrix, which is interfaced with a digital minicomputer, a teletypewriter, a magnetic tape unit, and a plotter. The position of a photocell shaded by the light interception of a passing fish and the time of this event are computed and recorded, and the information is stored on a computer disk pack. These raw data and their sequence form the basis for computation of the position of the fish in the tank, its velocity, the magnitudes of turns, the lengths and orientation of the steps, the distances covered, and the frequency distributions of all these values over time.
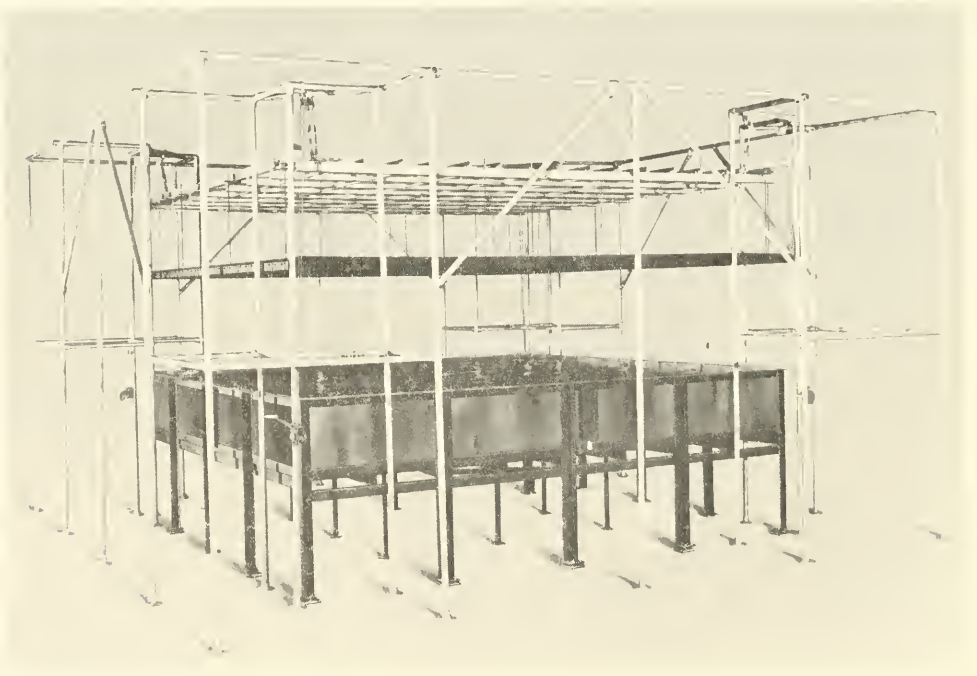
Figure 14   Shark monitor tank II; see Fig. 15 for explanation. From Kleerekoper (1969).
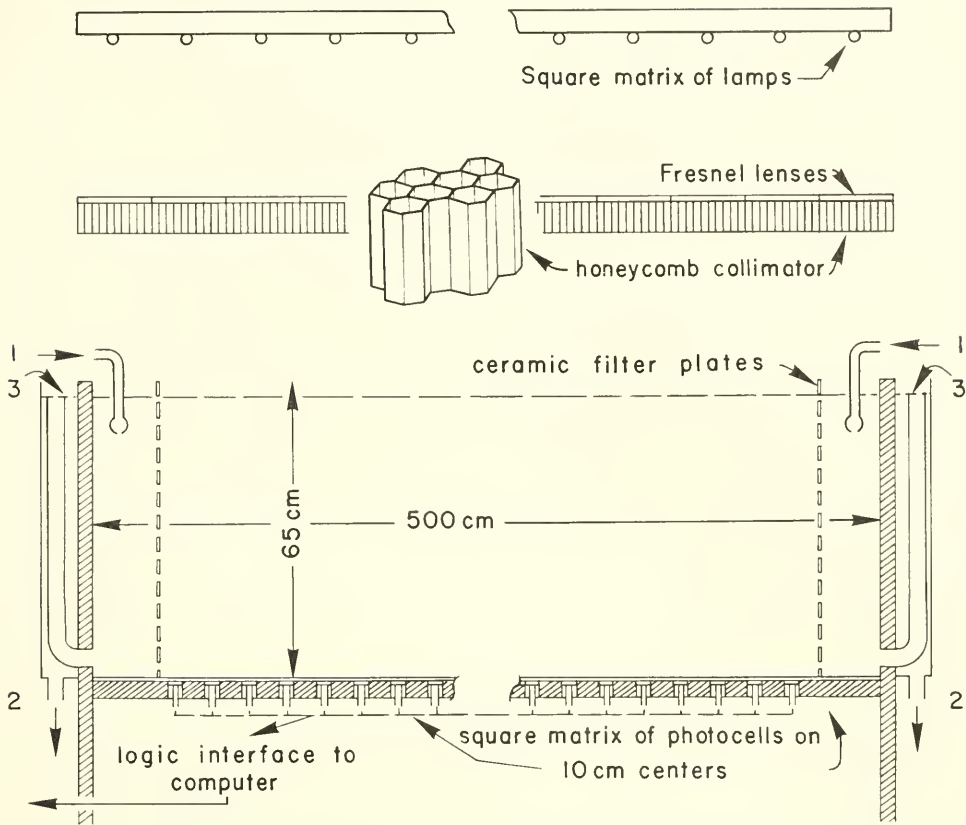
Figure 15 Shark monitor tank II. (1) Water supply into any one of four peripheral channels; the water enters the tank proper through a wall of ceramic filter material. (2) Return of water overflowing through standing pipe (3). The light from the square matrix of lamps suspended over the tank is collimated through a continuous layer of Fresnel lenses resting on a flat black honeycomb of aluminum foil. From Kleerekoper et al. (1969).

Various computer programs allow analysis of many statistical relationships among the variables and their behavior over time, not only in the monitor tank as a whole but in restricted areas, which can be specified.

Almost perfect laminar flow is obtained by admitting supply water into the tank through the entire area of one of the tank walls (Figure 15.1), made of ceramic filter plate and allowing it to exit through a similarly built opposite wall. This permits creation of discrete, controllable distributions of chemical solutions in the water so that the locomotor behavior of a fish can be analyzed as a function of the chemical condition prevailing in any area.

The release of stimuli at discrete loci and the establishment of chemical "trails" are made possible by 200 blunt hypodermic needles, embedded flush in the floor of the tank, in a square matrix on 20-cm centers. These needles are individually connected to a container through silastic tubing and can be made to release, singly or in any desired combination, simultaneously or·in

sequence, solutions stored in the container, at controlled rates. Thus, release of a chemical trail can be established "upstream," "downstream," parallel or at angles with the stream, in stagnant water or in currents of various flow rates.

The above outline of the experimental approach to the analysis of locomotor behavior in general and oriented behavior specifically, is to serve as the background for a discussion of some of the results obtained in this laboratory in the study of that behavior in some sharks.

OBSERVATIONS ON THE GENERAL LOCOMOTOR
BEHAVIOR OF THREE ELASMOBRANCHS:
SCYLIORHINUS, *MUSTELUS*, AND
GINGLYMOSTOMA

In the late fifties and early sixties, an extensive effort was made in the author's laboratory to identify the main chemical components of the "body odor" of trout and other teleosts (Kleerekoper and Mogensen 1959) olfactorily attractive to the sea lamprey, *Petromyzon marinus* (Kleerekoper and Mogensen 1963). One component, first code-named amine F, later identified as isoleucine methylester, when perceived in very low concentration by either adult or larval *Petromyzon*, elicited general and oriented locomotor activity, resulting in efficient localization of the stimulus source by olfaction only (Kleerekoper and Mogensen 1963, Kleerekoper 1963).

Later, a study was begun to verify the attractiveness of the substance to teleosts and elasmobranchs. Early in that study, by long-term monitoring of locomotion, it was established that, contrary to expectations, the spatial characteristics of the locomotor patterns of neither the elasmobranchs (*Scyliorhinus* and *Mustelus*) nor the teleost (*Diplodus*) under investigation were randomly distributed in the symmetrical environment of the cylindrical monitor, even in the absence of experimentally controllable cues (Kleerekoper 1967). Both elasmobranchs, because of biases in their locomotor behavior, displayed a preference for certain pathways in the tank. One of these was "handedness," that is, turning predominantly to either the right or the left on emerging from a compartment. In a typical experiment, *Scyliorhinus stellaris* might display a 1.6 ratio (n = 2056) and *Mustelus mustelus* a 0.6 ratio (n = 1492) of left/right turns. Obviously, such handedness alone strongly biases the locomotor pattern, but in addition both species, on emerging from a compartment, manifested a strong preference to bypass or "jump" a set number of compartments before making the next entry (Figures 16 and 17). That behavior, however, might be different following left or right turns, as in Figure 18, in which the distributions of the number of bypassed compartments are illustrated for *Scyliorhinus*. The differences between left and right turns were much smaller in *Mustelus* (Figure 19).

Numerous experiments, representing many thousands of events, confirmed, in the above species, nonrandom locomotor patterns, which could be accounted for almost entirely by the two biases mentioned. At the same
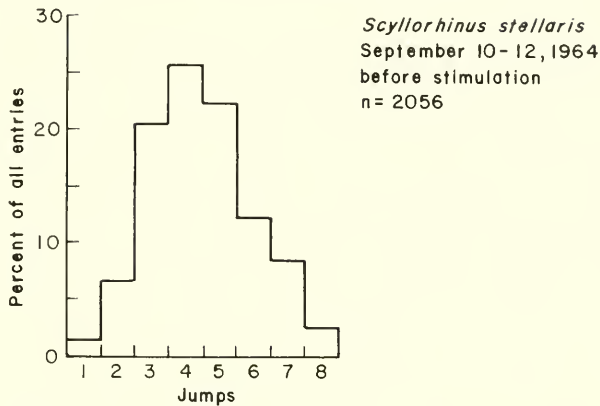
Figure 16 *Scyliorhinus stellaris*: frequency distribution of the number of compartments bypassed ("jumps"), as a percentage of all entries, prior to stimulation. From Kleerekoper (1967).
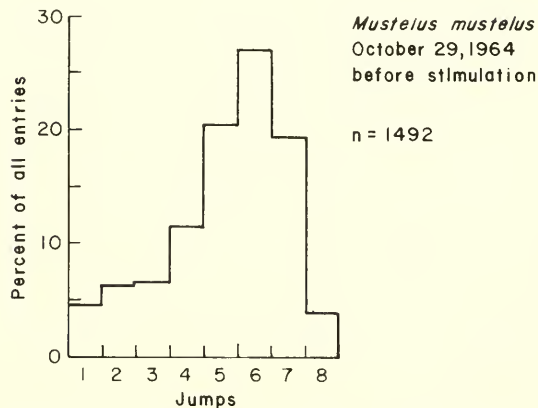
Figure 17 *Mustelus mustelus*: frequency distribution of the number of compartments bypassed ("jumps"). From Kleerekoper (1967).

time, they were also verified in the teleost *Diplodus sargus*, which has a highly pronounced handedness and jump preference, suggesting species specificity of the bias magnitudes. These results led to the postulate that locomotion, even when not oriented, is not the outcome of a random sequence of independent locomotor actions (turns, steps, velocities), unsystematic in their occurrence, magnitudes, and temporal relationships, but of an organized, systematic array of such actions under the control of the central nervous system. Initially, supporting evidence for the above postulate was sought in studies on the locomotor behavior of the goldfish by the advanced techniques afforded by the second monitor system (a square matrix of photocells). In a series of reports (Kleerekoper et al. 1969, 1970; Westlake
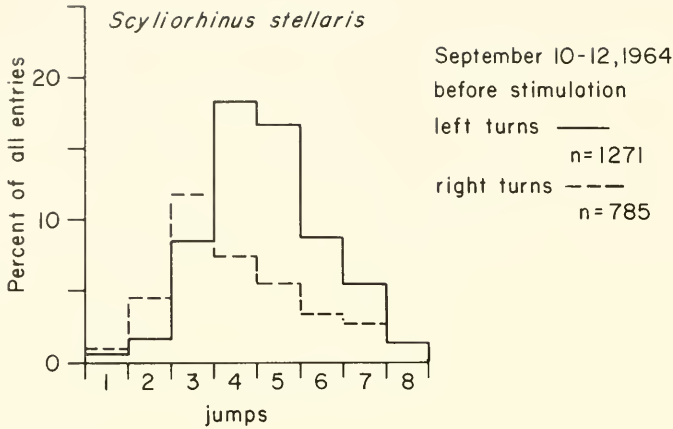
Figure 18 *Scyliorhinus stellaris*: frequency distribution of "jumps" for left and right turns, prior to chemical stimulation. From Kleerekoper (1967).
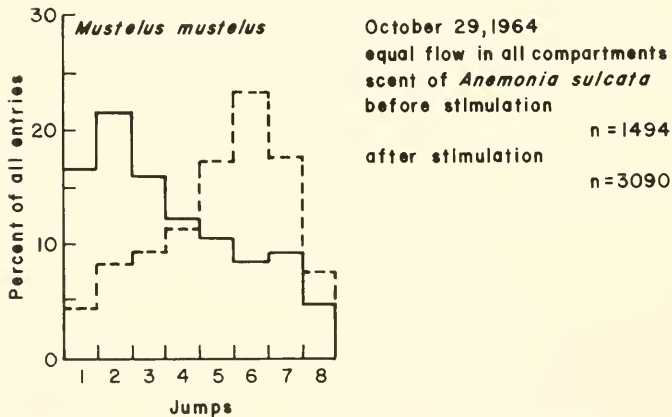
Figure 19 *Mustelus mustelus*: frequency distribution of "jumps" for right and left turns, prior to chemical stimulation. From Kleerekoper (1967).

and Kleerekoper 1970), considerable systematization of various locomotor variables could be demonstrated, but recognition of the exact nature of the resulting system awaited detailed time-series analysis of existing and new data.

   This was carried out under the direction of Dr. J. H. Matis, of the Institute of Statistics of Texas A & M University. These studies firmly established, and quantified to a considerable degree, the principle that locomotor behavior is determined in magnitude as well as occurrence over time, by a highly organized interdependence of locomotor variables (Matis et al. 1973; Matis, Childers, and Kleerekoper 1974; Matis, Kleerekoper, and Gensler 1974). The new insight made possible quantitative analysis of exploratory behavior

in the same species (Kleerekoper et al. 1974). With the analytical techniques well in hand, and the principle established in a teleost, quantitative analysis of the locomotor behavior of an elasmobranch, *Ginglymostoma cirratum*, was undertaken. Although this research is continuing, the results to date will now be discussed in some detail, based on published (Matis, Kleerekoper, and Childers 1974; Matis, Kleerekoper, and Gruber 1975) and still unpublished data (Gerald et al., in press).

The goldfish studies had demonstrated that locomotor behavior can be accurately described through the quantification in time and space of a number of key variables. Seventeen such variables were defined: (1) number of right turns, (2) number of left turns, (3) number of combined turns, (4) radian accumulation of right turns, (5) radian accumulation of left turns, (6) radian accumulation of combined turns, (7) difference between right and left radians, (8) mean size of right turns, (9) mean size of left turns, (10) mean size of combined turns, (11) mean difference between right and left turn size, (12) ratio of left turns to combine turns, (13) ratio of left radians to combined radians, (14) ratio of straight path to turns, (15) distance travelled (cm/s), (16) mean step length, (17) mean velocity (cm/s).

For each experiment, the record of photocell responses to the presence of the shark was converted into time series of 15-min intervals, and the data for each interval transformed into the 17 variables. It had been established that the time series of these variables have distinct patterns of serial correlation over time (Matis et al. 1973). The strength and the varying periodicity of the oscillations of the 17 series suggested the existence of a complex multivariate interrelationship that determines the constraints on locomotor behavior. These studies were expanded, including additional data, and it was demonstrated that only seven of the time series are sufficient for maximum locomotor predictability and could form the basis for a satisfactory locomotor control model (Matis, Childers, and Kleerekoper 1974).

Against this background of information on the goldfish, studies of the locomotor behavior of the nurse shark were initiated, with the immediate object of constructing a refined locomotor control model for a representative elasmobranch. To this effect, the time series behavior of the 17 locomotor variables was analyzed based on a large data set acquired in six experiments with three nurse sharks (Matis et al. 1975). The analytic approach had three objectives: to determine the frequency of serial correlation in the data, to calculate the proportion of residual variation remaining after fitting a first-order autoregressive equation, and to analyze the proportion of residual variation to ascertain whether it differed between experiments and variables. It was found that in this elasmobranch, as in the previously studied teleost, definite serial correlation occurs in the locomotor variables of all the animals and that the strength of this correlation varies among the sharks, and within the same shark between different experiments. The degree of serial correlation varies among the 17 variables, reflecting the strengths of the feedback structures by which they are controlled.

It is not yet clear to what the variations in the strength of the correlations must be attributed. However, it is speculated that the feedback mechanism

responsible for serial correlation may be responsive to sensory information, and hence to external stimuli. This assumption is supported by the fact that water flow affects the strength of the serial correlation, a fact which will be referred to again later. The degree of serial correlation differs among the 17 variables, which means that the strengths of the feedback mechanisms controlling these variables are dissimilar. By comparing residual ratios and means, it was established that some locomotor variables are subject to strong feedback control and may therefore be considered rigid while others are weakly controlled by feedback. It was hypothesized that the latter are therefore more readily modulated when the animal responds to the environmental stimuli that provide directional cues for locomotion.

It is notable that among the most weakly controlled variables are those related to turning behavior: step length and mean turn size. When the variables are ranked by the strength of the feedback that controls them, the rank order is rather consistent among the animals tested, in spite of great differences in the absolute strengths of the serial correlations. The stability of the ranking as well as other statistical properties strongly suggest the existence in elasmobranchs of a relatively rigid locomotor control mechanism. Gerald et al. (in press) greatly extended the scope of the analysis of *Ginglymostoma*'s locomotion by describing the structure of the internal time dependency within each of the locomotor time series and by constructing a model of lesser dimensionality and internal dependency. Another feature of that research, closely tied in with the above, will be referred to later in discussing locomotor forecasting techniques.

For the quantitative aspects of this and the previous study the interested reader is referred to the original communications (Matis, Kleerekoper, and Gruber 1975; Gerald, Matis, and Kleerekoper, in press). A comparative study, in progress, of the above properties of locomotor variables in an elasmobranch and in a representative teleost (goldfish) may shed light on evolutionary aspects of locomotor control in fishes.

LOCOMOTOR RESPONSES TO CHEMICAL STIMULATION

General responses

In *Scyliorhinus*, stimulation with the odor of a conspecific in one of the 16 compartments of monitor I did not affect the relative frequency of movements between compartments but did have a sustained effect (90 min) on the distribution of "jumps" (number of compartments bypassed between egression and ingression) as well as the relative participation of right and left turns in this behavior as shown in Figures 20 and 21 (Kleerekoper 1967a, b). In other words the frequency distribution of turn sizes as well as the role of handedness in locomotion were affected. That the quality of the olfactory stimulus does not affect the mode of the response mechanism appears from the results obtained when *Mustelus* was stimulated not with food odor but with the odor of *Anemonia sulcata*, a noxious organism. The compartment releasing the odor substance as well as five of the neighboring compartments
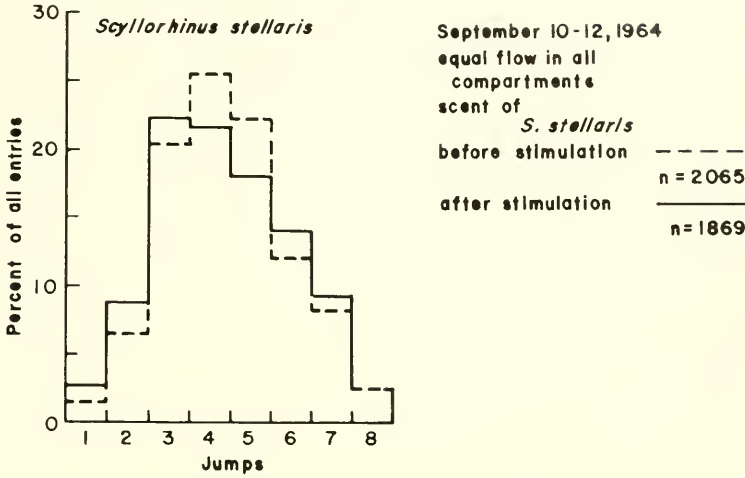
Figure 20 *Scyliorhinus stellaris*: stimulation with the odor of a con-
specific significantly changes the frequency distribution of the
"jumps." From Kleerekoper (1967).
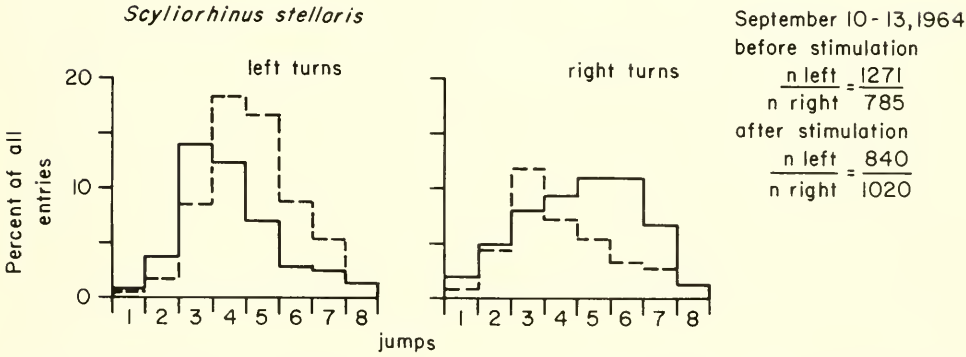
Figure 21 *Scyliorhinus stellaris*: left and right turns are affected differently as the animal
responds to the odor of a conspecific. From Kleerekoper (1967).

were significantly avoided. Analysis of this response shows again that the
locomotor variable involved is that of turning behavior. Similar response was
described for the teleost *Diplodus* in the same experimental arrangement.
The modifications in turning behavior were not restricted to the cases in
which actual localization of the source compartment occurred but were
manifest also in the absence of accurate orientation.

    In all these experiments, olfactory stimulation resulted in a decrease of
the mean angle of turns which, in the peculiar spatial design of the monitor,
resulted in an increase over time of the number of different compartments
entered by the animals. In the absence of the dividers, that is, in an "open
field," the behavior would have resulted in a more intensive coverage of the
area and might have been called "searching." The results of the analysis of

locomotor responses of *Scyliorhinus*, *Mustelus*, and *Diplodus*, the frequent references by earlier workers to responses involving turning movements, and the theoretical implications of the weak feedback control of turning-related locomotor variables strongly suggest that perception of biologically significant odors modulates mainly a turning control mechanism and thus changes the locomotor pattern. These effects were apparent whether or not the fish received directional cues from the stimulus source. They occurred even when the odor was admitted with the general water supply, thus equally affecting all 16 compartments of the monitor tank (Kleerekoper 1967b). None of the responses mentioned were observed in anosmic fish. That such modulation of turning behavior can also be effected by changes in water flow rates will be demonstrated below.

Oriented Locomotion

In a different experimental arrangement, the locomotor patterns of *Ginglymostoma cirratum* were cinematographically analyzed, in response to a discrete source of water to which extract of fish flesh had been added (Kleerekoper 1967a, b). The locomotor pathways of the shark, as it approached the source, frequently had spiral configurations, many of which were analytically shown to be parts of logarithmic spirals (Kleerekoper et al. 1973). In such an equiangular spiral, the lengths of radii of 90° have a ratio of 1:0.618 (Thompson 1942). In various organisms, suppression of sensory input of a single modality brings about spiral movements of the helical type. This has been shown for amoeba (Nägeli 1860; Jennings 1901; Schaeffer 1920, 1926), ciliates (Bullington 1925), tadpoles (Streeter 1906), dogfish (Lee 1894), and others, including man (Schaeffer 1928). In nurse sharks with two functional nostrils, the best logarithmic spiral fits were obtained in animals stimulated with odor diffusing into stagnant water. In such conditions, centripetal movements along the spiral predominated, in contrast to locomotion in the absence of olfactory stimulation or when one nostril was occluded. The records showed that such centripetal locomotion led the animals to the odor source.

The orientation mechanism instrumental in this behavior is suggested by the consideration that an animal approaching a target in a direct path, which always deviates with a constant angle from a straight line connecting the animal with the target, must follow a logarithmic spiral. In other words, locomotor orientation using a directional sensor system based solely on a bilateral input of constant differential magnitude will manifest itself in a logarithmic spiral locomotor pathway. The differential input may originate in the bilateral olfactory sacs; provided that the difference in strength of the stimulus affecting them is constant, the means of orientation by this principle are present. It was proposed that this is, indeed, the mechanism employed by the nurse shark, in the strong gradient prevailing in the experiments described. Bilaterally different olfactory stimulation must depend on the slope of the odor concentration gradient, which is steep in the vicinity of the source but rapidly flattens, through dilution and dispersion, as the

distance from the source increases. Given the proximity of the nostrils in all but a few fishes, a bilateral differential in stimulus strength, dependent on differences in molecular density of the odor substance, is an unlikely orientation mechanism at best, in all but the steepest gradients. In most of the natural environment, therefore, whenever olfaction is involved, a different orientation principle must prevail. The strongest biological evidence in favor of this postulate is the highly efficient olfactory orientation in *Petromyzon marinus* (Kleerekoper and Mogensen 1963), although, as a cyclostome, it has a single median nostril (monorhinic).

The results of earlier experiments (Kleerekoper 1967) suggested an orientation mechanism in which the role of olfaction would be unrelated to the gradient of the odor substance. It was observed that the teleost *Diplodus sargus*, when stimulated with an attractant odor, modified its turning behavior as described above and oriented toward the odor-releasing compartment of the tank, but was unable to localize that compartment. The fish generally entered the neighboring compartments as if it were turning "too early." In these experiments, the slow rate of water flow, from the periphery toward the center, was the same in all compartments (1 l/min). When, however, a differential flow was created increasing the rate in the odor-releasing compartment by 10%, localization by the fish became absolutely accurate, although no preference for that compartment occurred in response to the differential flow rate alone, that is, in the absence of the olfactory stimulus.

These results strongly suggested a similarity with the behavior of various unrelated organisms, described in the literature, in which odor acted as a releaser of rheotaxis. This phenomenon was first observed in the aquatic snails *Alectrion* and *Busycon* by Copeland (1918) and confirmed by Henschel (1932) for *Nassa reticulator*. Odor stimulation in stagnant water evoked random "alarm" movements but when a current was produced, locomotion became at once positively rheotactic. Similar behavior has been observed in terrestrial organisms that display anemotaxis when stimulated with an attractant odor, such as *Triton* (Czeloth 1931), *Drosophila* (Flügge 1934), and *Planaria* (Doflein 1926).

The significance of the findings with *Diplodus*, later confirmed in a series of experiments, is in the fact that a diffusion field of a chemical source contains no direction vector that might be used in orientation toward the source. Localization could only occur by random sampling and a topographic comparison of relative densities of odor molecules, the results of which would have to bias locomotor behavior in the general direction of increasing odor concentration. One such chemotropotactic orientation mechanism, applicable in a steep molecular gradient such as prevails at short distance from the odor source, was already discussed. However, it could not function, as was pointed out, beyond relatively short distances from the source. Water flow, on the other hand, contains a strong direction vector to which rheotactic movements respond in a highly efficient fashion. Hence, it was postulated that, in fish, the perception of an attractant odor, rather than allowing efficient oriented locomotion, merely releases positive rheotaxis (Kleerekoper 1967, 1969). In an odor-dependent displacement, be it in

migration, search for food, mate, or chemical quality of the medium, the animal would swim upstream as long as the chemical identity of the rheo-taxis-releasing odor (single substance or mixture) was perceived.

Because in such an orientation mechanism odor molecule densities are irrelevant as long as the threshold for olfactory stimulation is reached, the theoretical difficulties inherent in the "gradient search" hypotheses, pointed out by several investigators (von Buddenbrock 1952; Precht 1942; Otto 1951), are eliminated. In view of the significance such a mechanism may have in the orientation behavior of elasmobranchs, a series of experiments was designed to verify the role of interaction between olfaction and water flow in the ability of these animals to localize an attractant odor source, and to determine some of the quantitative relationships between these stimuli.

LOCALIZATION OF AN ODOR SOURCE BY *GINGLYMOSTOMA* AS A FUNCTION OF WATER FLOW

In this study (Kleerekoper, Matis, and Gruber 1975), the locomotion of single sharks was analyzed, by means of monitor II (square matrix of sensors), during 24-h control periods, in both flowing (1.17 cm/s) and stagnant water. Following this, and without interrupting monitoring, the chemical stimulus (dilute, filtered extract of mashed shrimp) was applied through needle no. 1 (Figure 22) embedded in the monitor's floor, at a constant rate (5.0 ml/min), during 2 h. At the end of this period, delivery was stopped while, in the experiments with flowing water, water continued to flow through the tank during at least 3 h; in the experiments with stagnant water, circulation was reinstated to allow filtration and to eliminate shrimp substance from the tank. This procedure was repeated five times with five different needles, as illustrated in Figure 22, which also indicates the direction of water flow, when present, and the quadrants of the tanks.

For an understanding of the results of these experiments, the following explanation seems pertinent. The monitor system yields, it may be remembered, a time series record of photocells, in a 44×44 matrix, triggered by the shark in the course of its locomotion. Data from each quadrant of the tank were analyzed separately, converted into 15-min intervals, and transformed into seven locomotor variables: (1) number of events (photocell triggerings), (2) distance traveled, (3) mean velocity, (4) mean turn size, (5) mean step length, (6) time spent in the quadrant, and (7) frequency of turning.

These data were then standardized. A distribution of the orientation angles of the steps (or straight pathways between turns) in each quadrant, for each period, was characterized by an angular mean vector with direction θ and strength r, adjusted for activity level. The resulting direction vector index was the eighth time series in the analysis of the response in each quadrant. Polynomial regression equations were calculated for each experiment, relating the mean response of each variable to the six nearly equidistant sources of stimulation to determine those variables, by quadrant, that

were significantly affected by the changing location of the stimulus. The effect of flow was tested for individual sharks by comparing the mean responses of the variables in one experiment with flow to the mean responses in another experiment without flow. Interaction between flow effect and stimulus location was determined and a polynomial regression was calculated to establish the significance of that interaction.

In a representative experiment with continuous flow, the total distance travelled by the shark during 15-min intervals, in the tank as a whole, decreased for the first 24 h, after which the level stabilized and increased again after the first 48 h, as illustrated in the time series of Figure 23, which indicates along the abscissa the times and places at which stimulation was applied. In Figure 24 these same data are presented for quadrants 1 and 4, as percent of total distance traveled. During the control phase, there is an initial "exploratory" period, recognizable in all four quadrants, during which the shark moves about the whole tank. Whether this "exploratory" behavior has spatial and temporal characteristics similar to those displayed by goldfish (Kleerekoper et al. 1974) has not yet been determined. After this initial period, the animal confined its movement almost entirely to the upstream
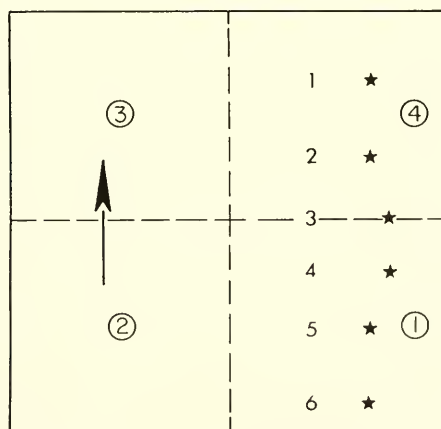
Figure 22 Disposition of the needles in the floor of monitor tank II, used to release for chemical stimulus. The solution or (for control purposes) seawater is kept in a plastic container to which all the needles are connected with surgical tubing. The rate of flow can be adjusted by raising or lowering the container, which is suspended with rope and tackle. Fine adjustments can be made with Hoffmann clamps. The rest of the needle matrix available for stimulation is not shown. Some of the monitor data were analyzed for the tank as a whole, and others for the different quadrants (indicated by circled numbers).
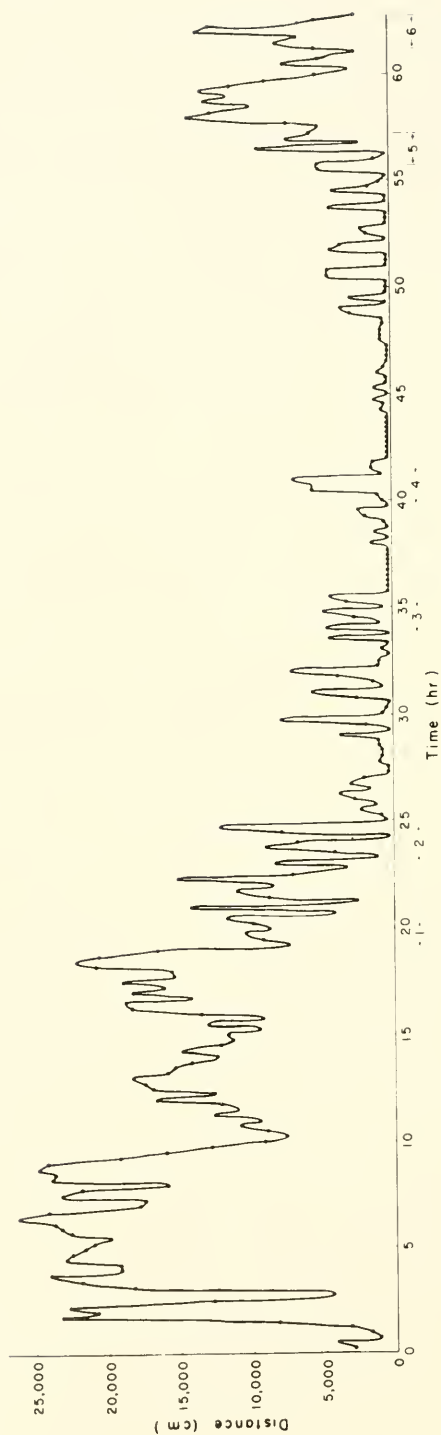
Figure 23 *Ginglymostoma cirratum*: time series of total distance traveled in the monitor tank as a whole. Numbers 1 through 6 along the abscissa indicate the needle through which the chemical stimulus is released. Only one needle is used at a time, and the duration of the release is marked by the horizontal arrows. Water flowed at 1.17 cm/s. From Kleerekoper et al. (1975).
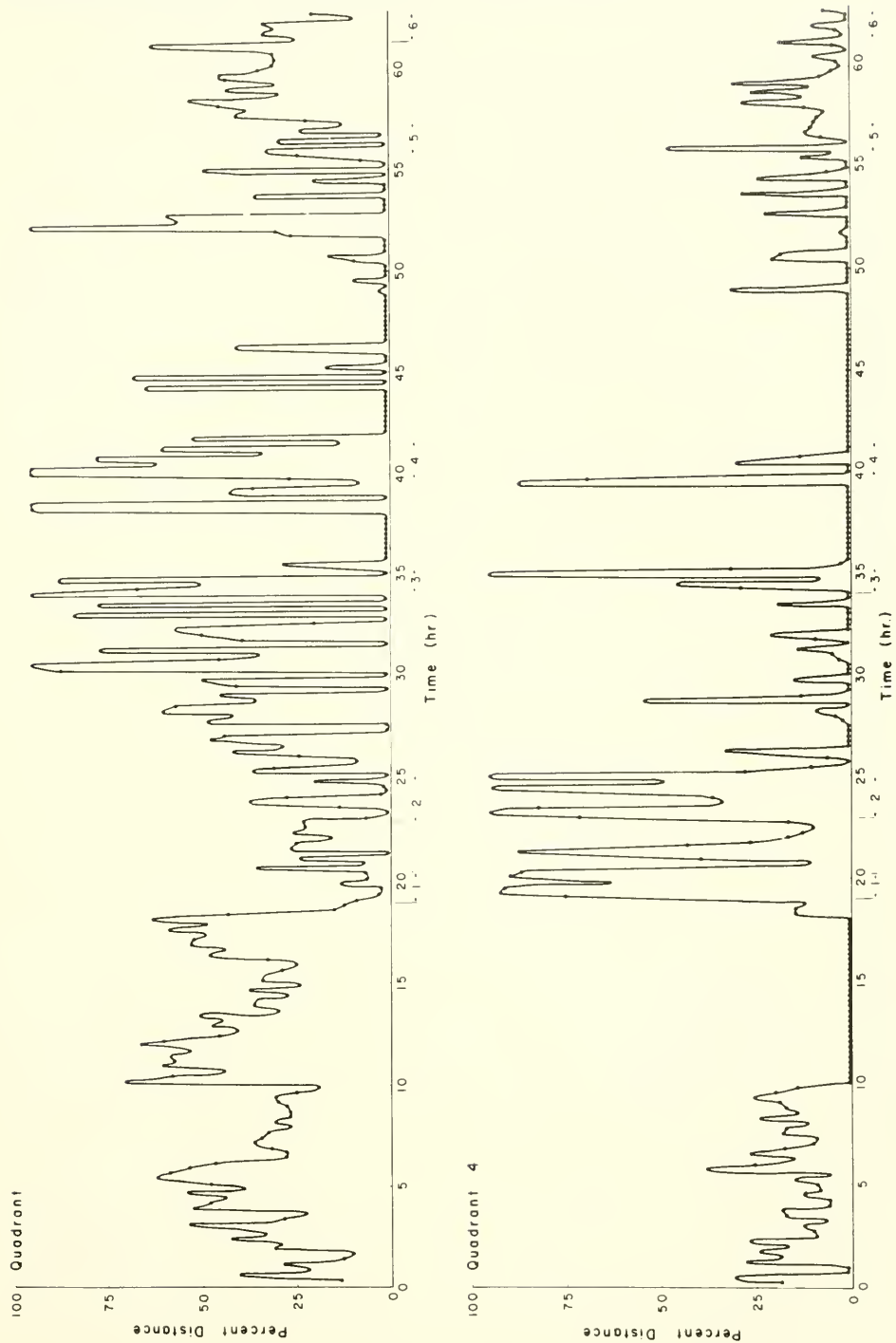
Figure 24   Data from Fig. 23, but for quadrants 1 and 4 only. From Kleerekoper et al. (1975).

quadrants 1 and 2. At the first stimulation through needle 1, quadrant 4, the shark responded immediately by restricting its movements almost entirely to that quadrant. During the following control phase (no stimulation), the previous upstream activity was gradually resumed. Throughout the remainder of the experiments, the alternation between periods of stimulation and control produced statistically highly significant (a = 0.001) responses, which, though similar, were quantitatively different as a function of the location of the stimulus.

The relationship between the percent of distance traveled and the six sources of stimulation was determined by regression analysis and modeled by a polynomial regression of up to second order. All the resulting regressions (Figure 25) were significant (a = 0.05) and quadratic except for the linear equation of quadrant 2. It is important to note that stimulation of site 1 (the most downstream location), in quadrant 4, resulted in nearly 90% of the distance covered by the shark to be in that quadrant. As the site of stimulation moved upstream, the distance covered in quadrant 4 decreased rapidly and that in upstream quadrants 1 and 2 (and to a much smaller degree in quadrant 3) increased.

The relationship between the response in direction vector and the site of the stimulation is of particular interest. As illustrated in Figure 26, release at site 1 (the most downstream location) resulted in a low directionality measure in each of the quadrants. As the stimulation site moved upstream, the statistic increased in the two upstream quadrants, with the response in quadrant 2 exceeding that in quadrant 1 for all sites. No such increase occurred in the downstream quadrants. By applying the above analytical procedures to the. data recorded from the experiments in stagnant water, the regressions illustrated in Figures 27 and 28 were obtained. In interpreting the above results, it should be remembered that the analyses refer to localization by the shark of the quadrants in which the release sites are situated, and not to the exact positions of those sites. The results, therefore, demonstrate the general localization of the attractant stimulus rather than the exact localization of its source, which will be discussed later.

The analyses showed that of all the variables studied, distance traveled, direction vector, and turning frequency are the most valuable in the analysis of the locomotor behavior involved in general localization. In flowing water, the regressions of percent of distance traveled demonstrate a fine distinction between adjacent release sites in downstream quadrant 4 (sites 1, 2, and 3), although some clear discrimination occurred also in upstream quadrant 1, which contained sites 4, 5, and 6.

The behavior of the direction vector further demonstrates the precision of the localization. A tight swimming pattern in the quadrant of stimulation (site 1, quadrant 4) is characterized by low vector values accompanied by high values for distance traveled (Figures 25 and 26). As the sites of stimulation move upstream, the locomotor pattern loosens, until at site 6 the pattern spreads beyond quadrant 1. The fact that the regression curves of quadrants 1 and 4 are not mirror images demonstrates that precise localization of the stimulus source is not exclusively dependent on the strength, rate of
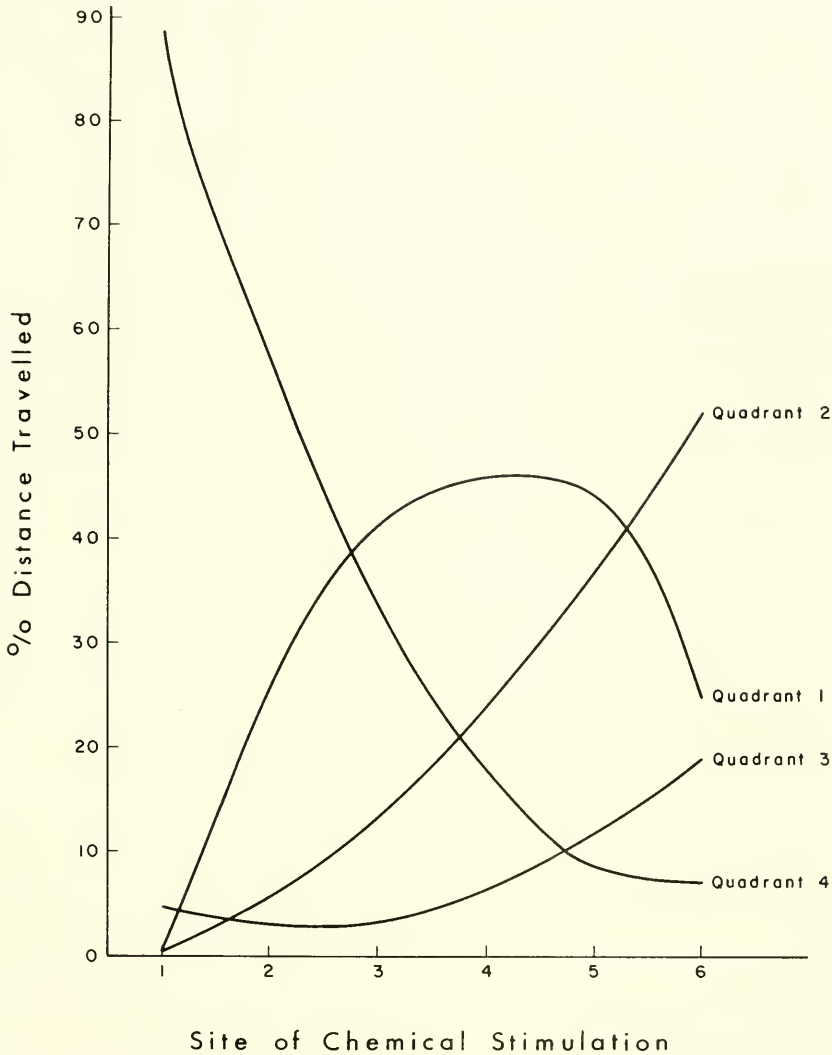
Figure 25 Data from Fig. 23: regressions of the percentage of distance traveled in the four quadrants of the monitor, as a function of the position of the needle releasing the chemical stimulation. From Kleerekoper et al. (1975).

delivery, and gradient characteristics of each individual site. The evidence presented above shows that the distance between the release site and the wall through which the tank exits is what determines the differences between the two curves. The greater that distance, the greater the dispersion of the stimulus and the shallower its gradient.

In stagnant water, the statistical relationship between the positions of sites 1 and 6 and the percent of distance traveled in the quadrants has a reduced linear slope. That is, it is less sensitive than that in flowing water and there is
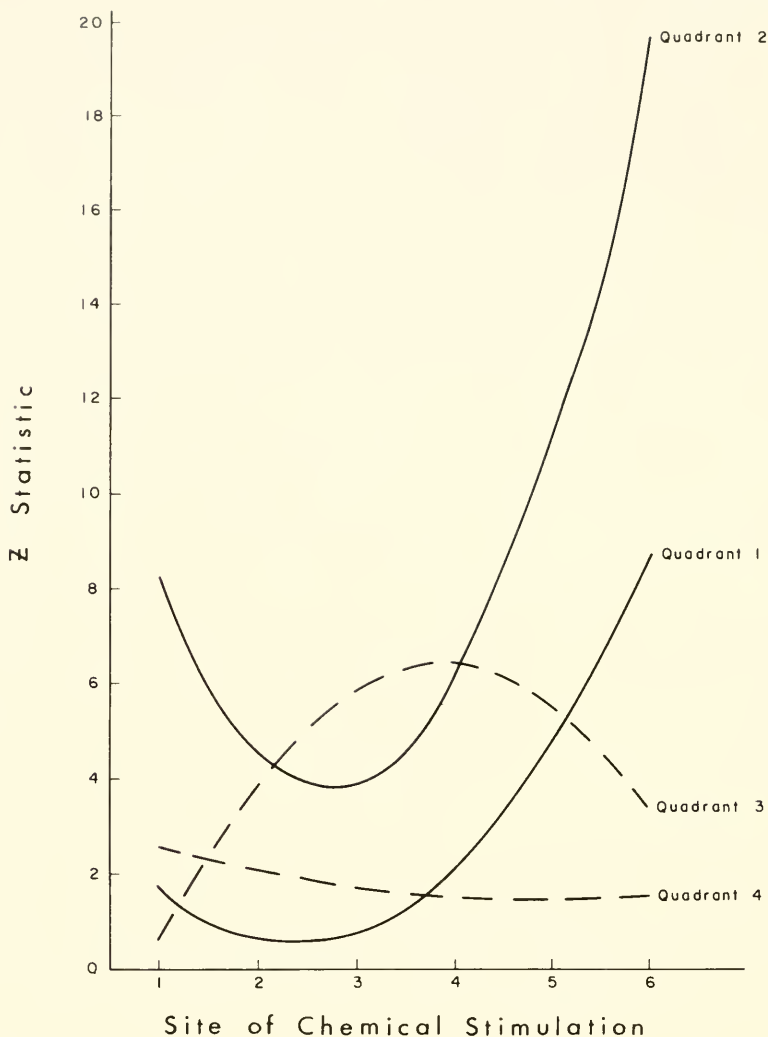
Figure 26   Data from Fig. 23: regressions of the statistic representing the direction vectors of steps between turns. From Kleerekoper et al. (1975).

greatly diminished discrimination of the release sites. This is evident also from the behavior of the values for the direction vector. The relationship between this vector and percent of distance traveled indicates that although the shark's locomotor pattern centered about the release site, its radius exceeded a quarter of a tank.

A summary of the differences of all six locomotor variables in flow and nonflow conditions is presented in Table 1 for quadrants 1 (upstream) and 4 (downstream). The table clearly shows that all the significant differences occur downstream (quadrant 4), where the gradient is strongest. This leads
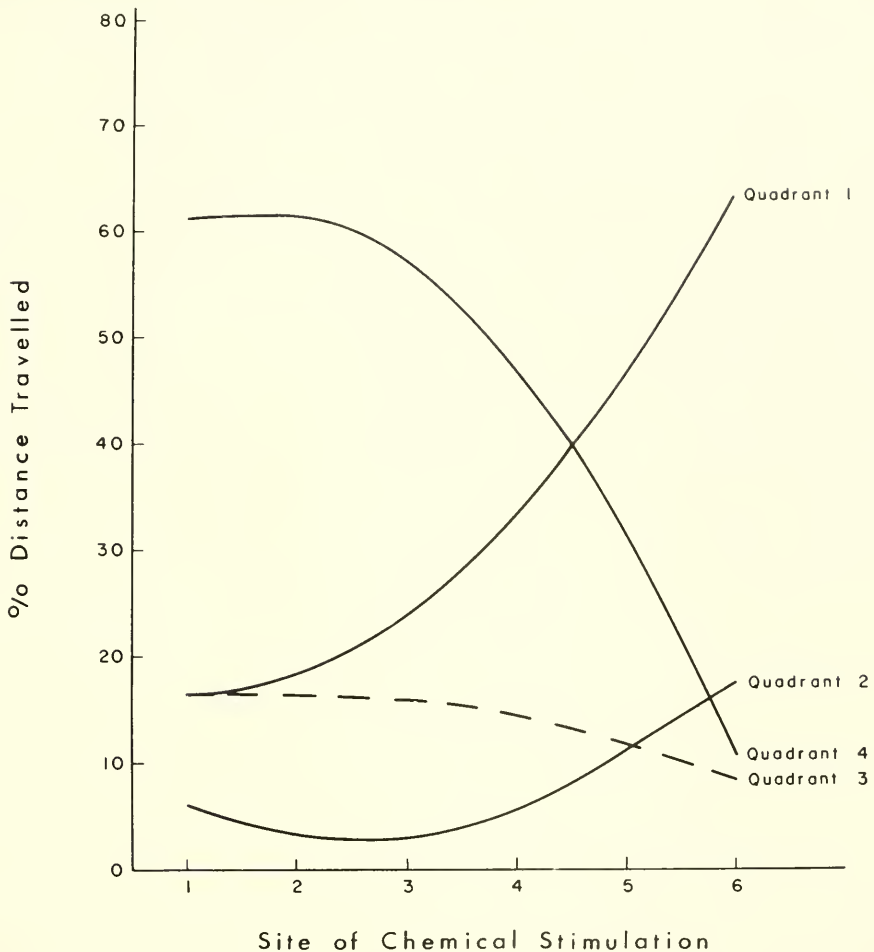
Figure 27 *Ginglymostoma cirratum*: regressions of the percentage of distance traveled in each of the quadrants of the monitor, as a function of the position of the needle releasing the chemical stimulation. Conditions were as in Fig. 25, but the water was stagnant. From Kleerekoper et al. (1975).

to the conclusion that the strength of the chemical gradient is important in determining the nature of the locomotor response and the accuracy in localizing the source of a chemical stimulus.

The mechanism for localizing the stimulus source is different in flowing and in stagnant water, as can be inferred from the turning behavior of the shark in the course of orienting toward the stimulus source in the two conditions (Figures 29a, b). The relationship between turning frequency and position of release site actually reverses with stimulation at site 1 and quadrant 1 as flow conditions change. In conditions of flow, relatively few turns are made, whereas in the absence of flow, almost 41% of all turns are made there.
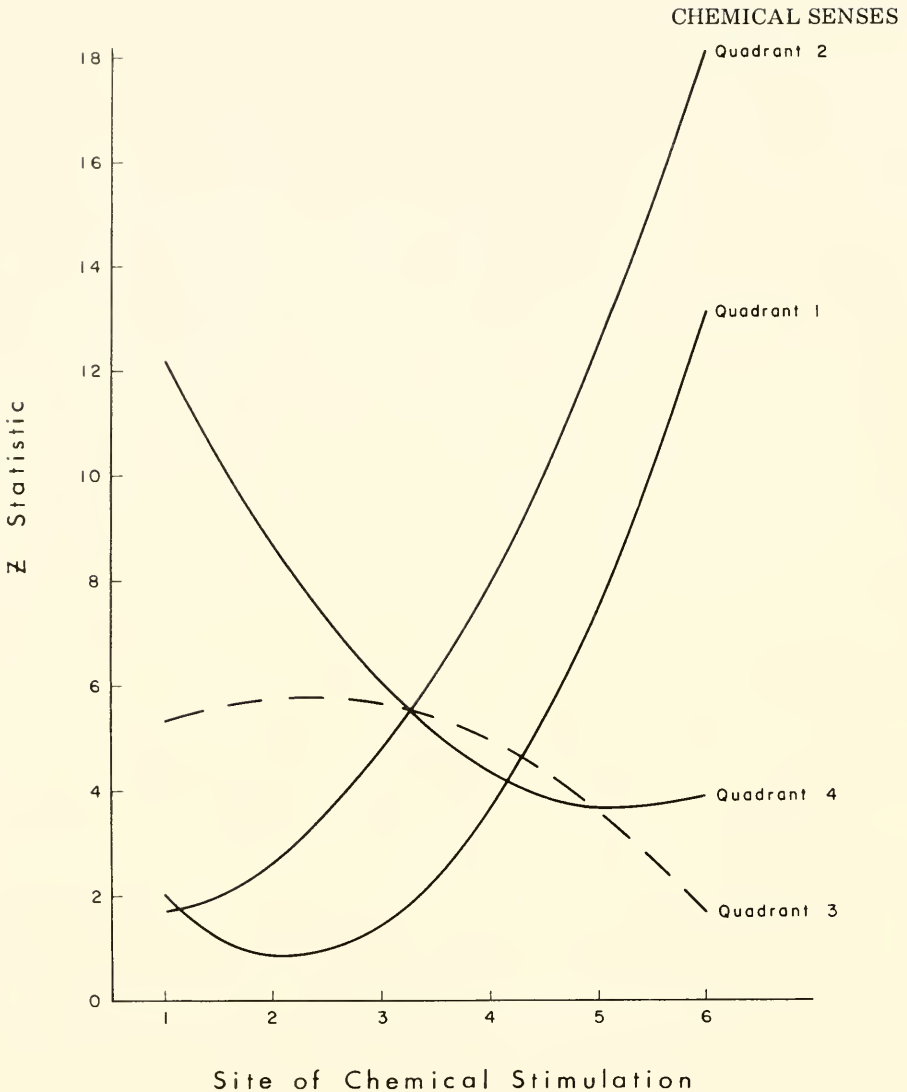
Figure 28   Regressions of the statistic. Conditions were as in Fig. 26, but the water was stagnant. From Kleerekoper et al. (1975).

   The shark's ability to pinpoint the source of stimulation is drastically affected by flow conditions, as time series of plots of its locomotor tracks demonstrate (Figure 30). The localization is precise in the slowly flowing water and requires only a few seconds to 3 or 4 min, depending on the position of the animal at the start of the release of the stimulus. The shark may remain exactly over the releasing needle for long periods, which are interrupted occasionally by short excursions in other areas, mostly near the stimulus source. Whether these excursions are movements in "search" of additional mechanical or visual information can only be speculated at this time. It is notable, however, that they are rare and of very short duration. Occasionally, snapping at the needle site has been observed.

Table 1. Differences resulting from flow and nonflow conditions of six locomotor variables in quadrants 1 (upstream) and 4 (downstream), as the shark responds to a single release site.*

Quadrant	Distance Traveled %	Events %	Time Spent %	Step Length %	Turns %	Velocity %
1	3.67	3.62	2.37	1.18	1.32	0.70
4	7.58[†]	8.56[†]	5.36[†]	6.24[†]	11.00[‡]	17.18[‡]

*Kleerekoper et al. (1975)
[†]$a = 0.10$
[‡]$a = 0.05$

In perfect concordance with the results of the time series and regression analyses, the precision of localization remains high for release sites 2 and 3 but decreases rapidly as the sites move upstream (4, 5, 6), although general localization of the area (quadrant 1) remains good. The possibility that these differences are due to habituation or adaptation can be ruled out, because precise localization of sites 1, 2, and 3 continues for the whole period of stimulation (100 min in the plots shown). The decreasing precision of localization of sites 4 to 6 must be attributed to the gradual decrease in spatial definition of the gradient as the chemical mixture moves downstream from the release site.

Behavior of the shark in stagnant water is strikingly different. Only exceptionally does it hover near the release site, and then only for a short time. Whereas the general area of release is localized by the animal, its responses are not discernibly related to the exact locale of the chemical source. This lack of precise localization is similar to that observed for sites 4, 5, and 6 in flowing water, and the cause may be the same, namely that such localization depends on a steep gradient. In a stagnant medium, the shrimp extract, on leaving the release needle, disperses and undergoes rapid dilution in all directions, resulting in a weak gradient. With flow (which is nearly laminar in the monitor tank), a downstream "corridor" of extract, with a strong mean direction vector, is formed. The conclusion to be drawn from these experiments is that "gradient search" is a very unlikely orientation mechanism in the natural environment, where gradients commonly are extremely weak.

A FORECASTING TECHNIQUE TO DETECT SUBTLE RESPONSES TO CHEMICAL STIMULATION

The experiments summarized above have demonstrated that fish frequently respond to environmental stimuli with subtle modifications of locomotor variables rather than with overt changes in swimming direction. Such modifications escape detection by direct observation not only because they are
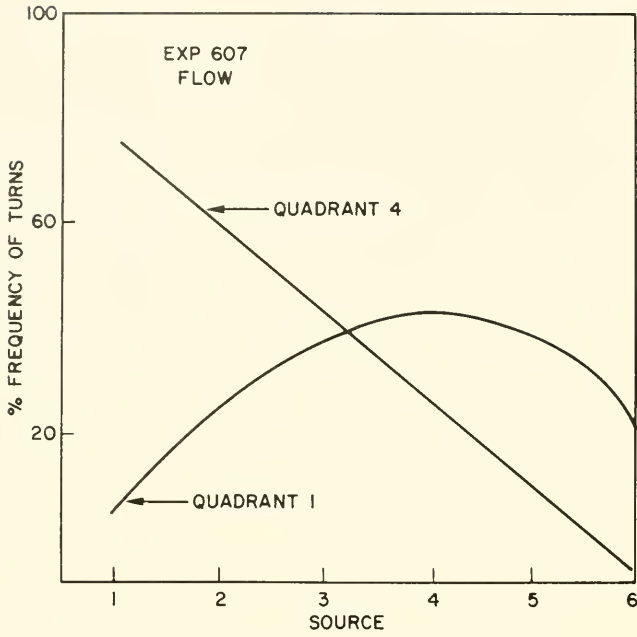
(a)   Flowing water.
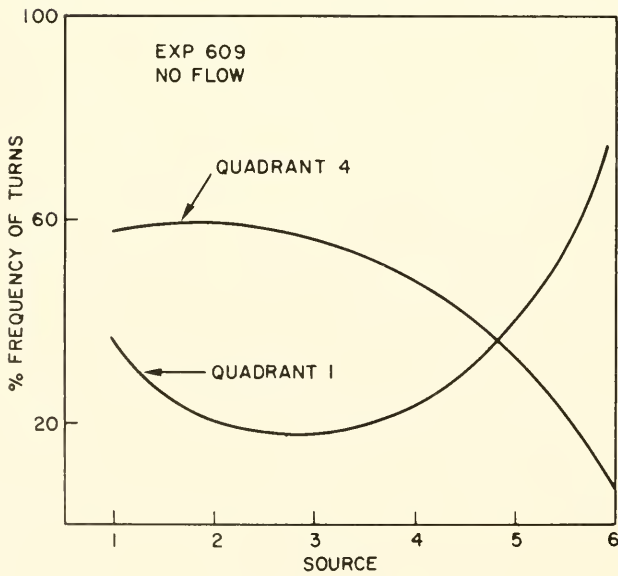
(b)   Stagnant water.

Figure 29   *Ginglymostoma cirratum*: frequency of turns vs
the site of stimulus release in quadrants 1 and 4 of the
monitor tank. From Kleerekoper et al. (1975).

Elapsed time in minutes after release of stimulus at · flow				
0-5	5-10	10-15	15-20	20-25
30-35	60-65	65-70	85-90	90-95
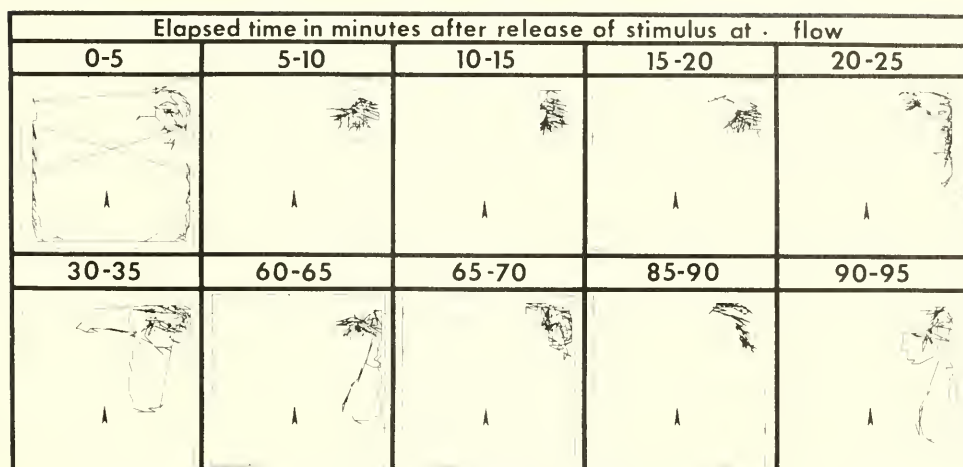
Ginglymostoma cirratum response to shrimp extract in flowing water (1.17 cms)

Elapsed time in minutes after release of stimulus non-flow				
0-5	5-10	10-15	15-20	20-25
30-35	60-65	65-70	85-90	90-95
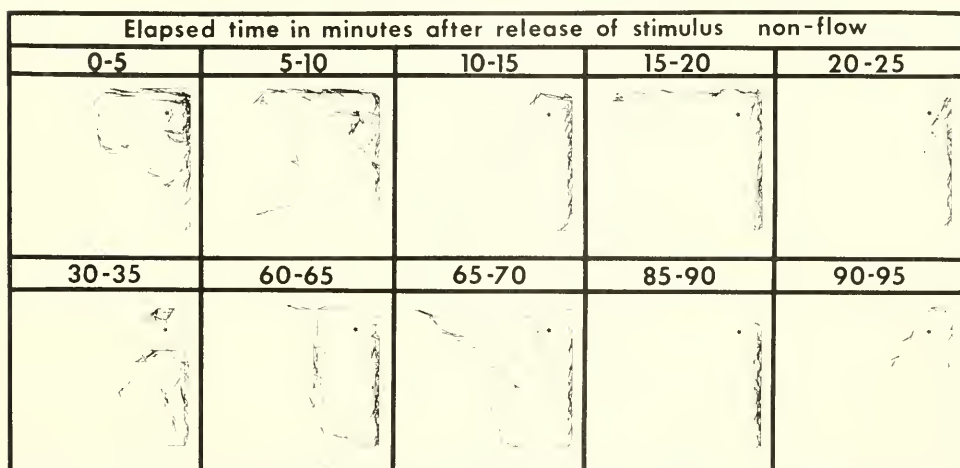
Ginglymostoma cirratum response to shrimp extract in stagnant water

Figure 30 *Ginglymostoma cirratum*: locomotor tracks plotted by computer, based on the same data as Fig. 23 (flowing water) and Fig. 27 (stagnant water), in 5-min time series. The stars indicate the position of the needle (#6) releasing the stimulus. The arrows in the top series indicate the direction of flow (1.17 cm/s). From Kleerekoper et al. (1975).

small but also because locomotor behavior is subject to great variability. Frequently the changes are spread out over time and therefore can be convincingly demonstrated only by long-term data acquisition.

In this laboratory, the question was raised whether such subtle responses to a stimulus might be detected more rapidly by determining the behavior of a locomotor model over time in constant conditions, forecasting its future behavior within acceptable limits of confidence, and verifying whether the behavior exceeds these limits as it is subject to the experimental stimulus being studied.

The concept was applied to nurse shark data before and during chemical stimulation, acquired by monitoring the locomotor behavior with the square matrix of photocells (Monitor II) (Matis, Kleerekoper, and Childers 1974). By the technique outlined above, two time series (mean velocity and mean step length) of 15-min intervals were obtained for a 17-h control period. Data from the initial, distinct, "exploratory" period were deleted. Gerald et al. (in press), in the analyses referred to above, using an improved model, applied the same concept to 17 time series of locomotor variables, of which Figure 31 illustrates the time series of mean velocity. The data on the left side of the graph, up to the arrow, refer to the control period of almost 20 h and were used to identify and estimate time series models for that period by the Box-Jenkins techniques (Box and Jenkins 1970), an approach necessary because of the distinct time series structure in the locomotor behavior of the nurse shark (Matis, Kleerekoper, and Gruber 1975), as mentioned earlier.

By the above procedure, discussed in detail in the original reports, a significant response by the nurse shark to a chemical stimulus applied at a
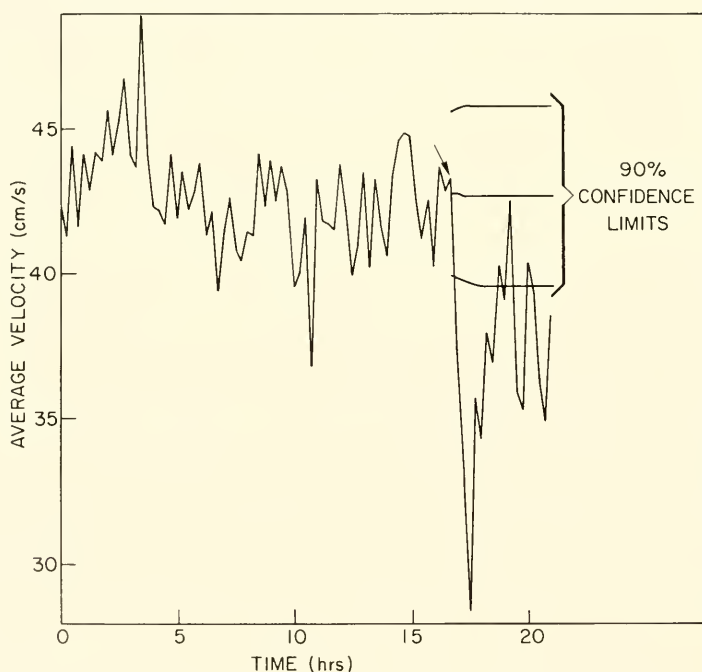
Figure 31   *Ginglymostoma cirratum*: time series of average veloc-
ities (cm/s). On the left side of the arrow is the control period
of 16.5 h, after which chemical stimulation was begun through
needle 6. The horizontally oriented curves indicate 90% con-
fidence limits of the forecast. At the point which stimulation is
begun the locomotor variable immediately "escapes from its
control"; the values of its time series greatly exceed the lower
confidence limit for several hours. From Gerald, Matis, and
Kleerekoper (in press).

point in the monitor's floor could be detected within one or two observations after application of the stimulus (Figure 31). Gerald et al. (in press) determined that chemical stimulation caused all 17 variables tested to "get out of control," thus manifesting the shark's response to the stimulus. This forecasting technique appears particularly promising as a highly sensitive diagnostic tool for determing the responsiveness of an animal to experimentally induced stimuli and their thresholds. The models are now being extended to the locomotor behavior of other species of elasmobranchs and teleosts, for use in detection of locomotor responses to various environmental variables. An important aim of these studies is to construct transfer models in which the input of a stimulus might be stochastically controlled to produce a desired output in the locomotor pattern. This, in turn, may lead to a quantitatively sensitive, cybernetic model of locomotor activity in these animals.

RESPONSES OF SHARKS TO FLOW

The important interaction demonstrated above between olfaction and flow in the orientation behavior of sharks, raised questions reqarding the quantitative relationship of these stimuli. For example, what are the effects of flow on locomotor behavior, in the absence of chemical stimulation? Do sharks discriminate between flow rates, and, if so, how accurately? What are the behavioral effects? Does the interaction of flow and odor depend on the flow rate?

In this laboratory, interest in these problems was heightened by the finding that short-term exposure to a subacute concentration of an acetylcholinesterase-inhibiting organophosphate (parathion) greatly affected the interaction between flow and odor in the orientation of the goldfish (Kleerekoper 1974; Rand et al. 1975; Rand 1976). Consequently, experiments were conducted on various aspects of flow response in nurse and lemon sharks (Gruber 1976; Maynard 1976). In the nurse shark, the average swimming velocity, based on data from 14 experiments, was significantly greater in flowing (1.17 cm/s) than in stagnant water in five of seven animals tested. Similar results were obtained with lemon sharks in two out of three experimental animals. The increase resulted from a shift in the frequency distribution of velocities: highest frequencies of high velocity classes in flow, of low velocities in nonflow conditions.

Regardless of these conditions, in lemon sharks the distribution of velocities depended also on the animal's position in the tank, a characteristic described earlier for normal (Kleerekoper et al. 1970) and blinded (Timms and Kleerekoper 1970) goldfish. For goldfish, it was established that the effect of position on velocity (and angle of turning) is determined by the fish's distance from the tank's walls, which have a significant effect on the locomotor behavior long before their position could interfere with the swimming of the fish. The fact that blinded goldfish behaved similarly strongly

suggested a role for mechanoreception, probably through the lateral-line system.

It is of particular interest that the nurse shark's locomotor velocity is not only affected by the proximity of a wall but that this effect is different in flowing and stagnant conditions. In flowing water, the ability to perceive the walls seems enhanced (Gruber 1976). This supports the assumption that the lateral-line system mediates the remote perception of barriers, in this case the tank's walls. In three nurse sharks, flow conditions affected also the step length, which increased significantly in stagnant water, based on data collected during experimental periods of longer than 20 h each. As to the angle size of turns, individual sharks displayed considerable variations with respect to this variable, and no generalization could be made. With respect to other locomotor variables, the effects of flow are summarized for both species of sharks in Table 2.

Maynard (1976) monitored the locomotor patterns of single lemon sharks first in uniformly flowing water (1.74 cm/s), in monitor II (square matrix of photocells), for 24 h. Next the flow pattern was arranged to have flow only in the central one-third "channel" of the tank. In three successive experiments the flow rate in that channel was adjusted to 1.75, 0.80, and 0.40 cm/s (Figure 32). The experiments were separated by periods during which there was no flow at all.

Typical responses to these conditions are illustrated in the tracings of Figures 33, 34, and 35. The animal restricted its locomotor pattern to the "channel," even at the lowest flow rate tested (0.40 cm/s), but the effect was maximal at the highest rate of flow. It required a few minutes to

1. UNIFORM FLOW

2. NO FLOW

3. CHANNEL — 1.75 cm/s

4. NO FLOW

5. CHANNEL — 0.80 cm/s

6. NO FLOW

7. CHANNEL — 0.40 cm/s
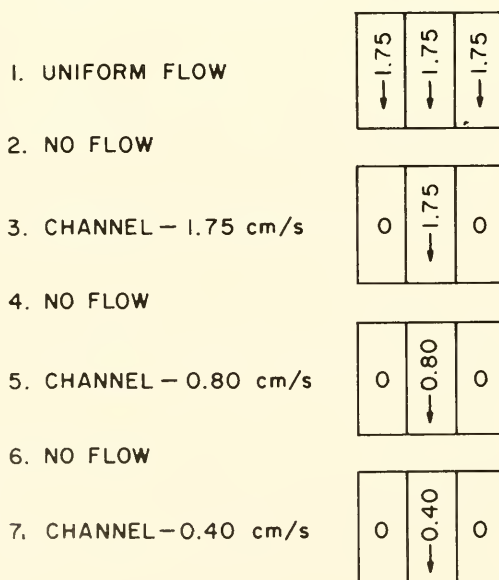
Figure 32   Schematic of experimental flow conditions in monitor tank II. From Maynard (1976).

Table 2. A comparison of locomotor variables in flowing (1.17 cm/s) and stagnant water for nurse and lemon sharks.*

Locomotor variable	Genus of shark	Number of sharks analyzed	Number of sharks displaying a change (as denoted in the following columns) for this variable	During flowing water conditions this change was (greater, less) than during stagnant conditions
I. Average velocity	Ginglymostoma	7	5	Greater
	Negaprion	3	2	Greater
II. Average step length	Ginglymostoma	7	4	Less
	Negaprion	3	3	Less
III. Frequency of turns	Ginglymostoma	7	4	Greater
	Negaprion	2	2	Greater
IV. Variance of the frequency distribution of velocity	Ginglymostoma	7	2	Less
	Negaprion	3	2	Less
V. Variance of the frequency distributions of step lengths	Ginglymostoma	7	5	Less
	Negaprion	3	2	Less
VI. Variability of the frequency distribution of angles	Ginglymostoma	4	Pooled data	Less
VII. Right:left hand turn ratios	Ginglymostoma	8	5	Ratio reversed

*D. Gruber (1976).
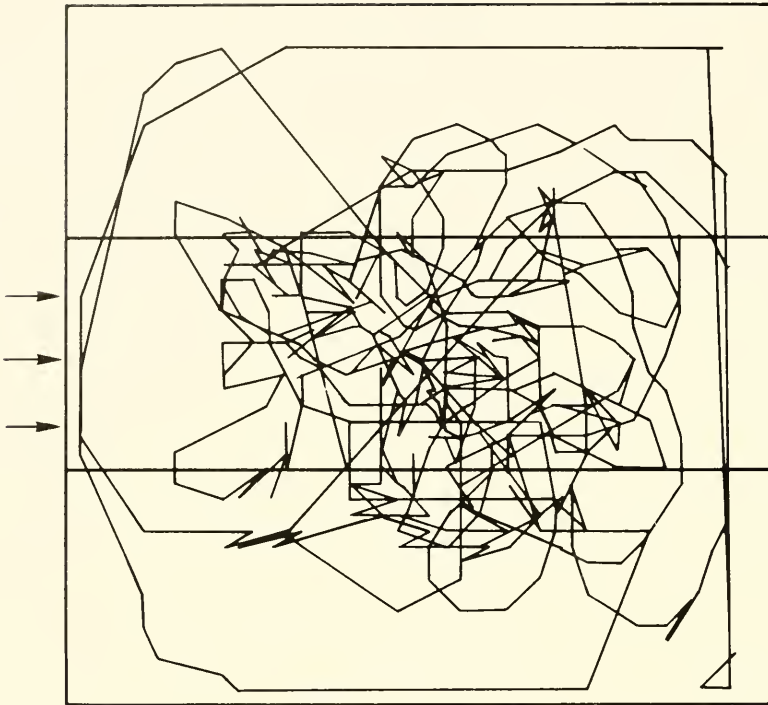
Figure 33 *Negaprion brevirostris*: locomotor track in monitor tank II
with water flowing only in the central "channel" at 1.75 cm/s, as
indicated by arrows. After Maynard (1976).

"tighten" the pattern after onset of the flow, as is evident from Figure 35, in
which the record of the first 12 min of another lemon shark is broken up
into periods of 3-5 min.

In parallel experiments, nurse and lemon sharks were tested in Monitor I
(cylindrical tank with 16 free-choice compartments) in which various flow
conditions were created in one or more compartments to ascertain the spon-
taneous locomotor responses to such conditions. Both species discriminated
flow rates at least as low as 0.1 cm/s, but they responded differently. The
nurse shark remained significantly longer in the compartment without flow,
whereas the lemon shark responded by more frequently entering compart-
ments with flow. With one exception, difference in behavior was consistent.
This surprisingly high sensitivity to flow in both species might be mediated
by the lateral-line, electroreception, or both systems in concert. The above
experimental approach, by monitoring locomotor behavior, provides a sensi-
tive test for the study of this aspect of the problem.

Gruber (1976) found that flow increased the swimming velocity of both
lemon and nurse sharks. Turning behavior was always affected by changes in
turning frequency and step length.
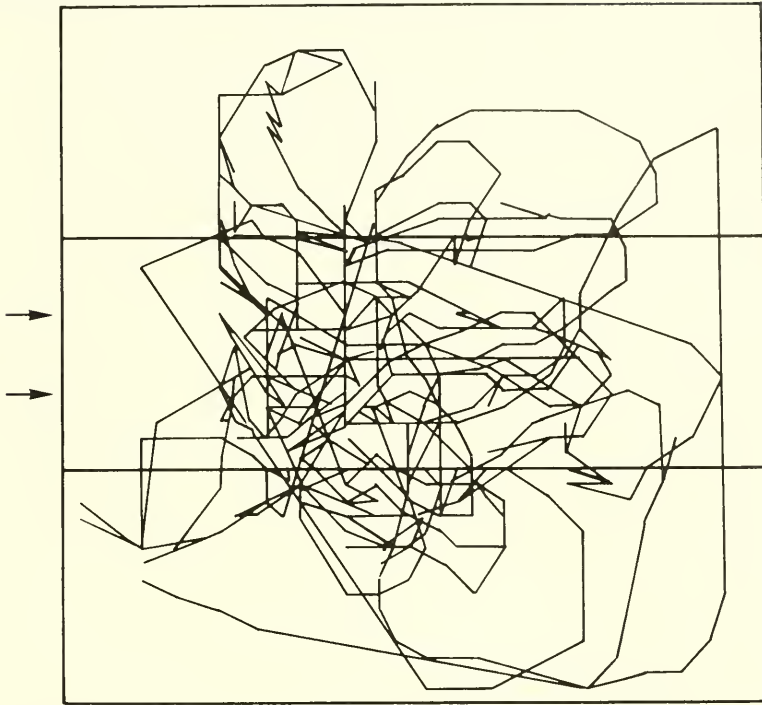
Figure 34   Locomotor track of *Negaprion brevirostris* under the conditions of Fig. 33, but with a flow rate of 0.80 cm/s. After Maynard (1976).

INTERACTION OF LIGHT AND CHEMICAL STIMULATION
IN THE EFFECTS ON THE LOCOMOTOR BEHAVIOR
OF THE NURSE SHARK

In this laboratory, Dorn (1976) exposed nurse sharks to four treatment combinations—(1) light, (2) chemical stimulus, (3) light and chemical stimulus, and (4) control—in the cylindrical monitor (I) and analyzed the interaction of the two stimuli (Steel and Torrie 1960; Ostle 1963). This shark was positively photoactic at a light intensity of 1.87 W/cm^2, and its behavior clearly demonstrated a significant interaction between the light and chemical stimuli, which means that the response to the combination of the stimuli differs from simple additive responses to the individual stimuli. Similar interaction phenomena have recently been described in other organisms and for various stimuli (Kleerekoper, Waxman, and Matis 1972; Kleerekoper, Westlake, Matis, and Gensler 1972; Rand et al. 1975; Vernberg et al. 1973), but not in sharks. The significance of such interaction in the orientation of these fish to chemical stimuli remains to be investigated in greater detail.
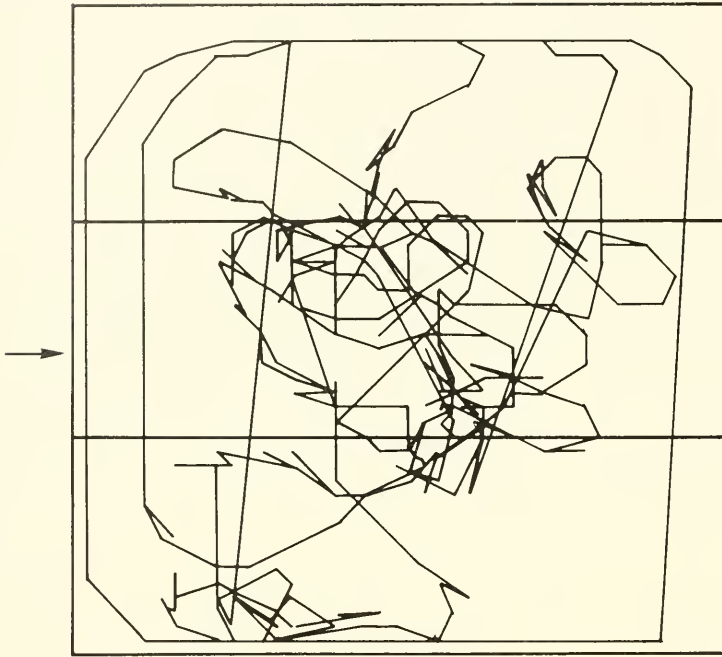
Figure 35 Locomotor track of *Negaprion brevirostris* under the conditions of Fig. 33, but with a flow rate of 0.40 cm/s. After Maynard (1976).

CONCLUSIONS

The overview of the "state of the art," presented here, reemphasizes the need for quantitative documentation of the locomotor responses of sharks to chemical and physical cues. The effects of behavioral interaction between stimuli of different modalities has barely been considered in the literature, although, in natural conditions, the animal is continuously bombarded by stimuli, the values of which are frequently interdependent. Evidence for the significance of interaction between chemical stimulation and water flow, as well as chemical stimulation and light, has been presented for elasmobranchs as well as teleosts. These interactions and their quantitative aspects deserve a great deal more attention. The effects of multiple stimuli impinging simultaneously are virtually unknown and their study presents a challenge which will have to be met if better insight into the responses of elasmobranchs (and other animals) to environmental information is to be gained.

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IV MECHANICAL AND ACOUSTICAL SENSES

MECHANORECEPTORS AND THE BEHAVIOUR OF ELASMOBRANCH FISHES WITH SPECIAL REFERENCE TO THE ACOUSTICO-LATERALIS SYSTEM

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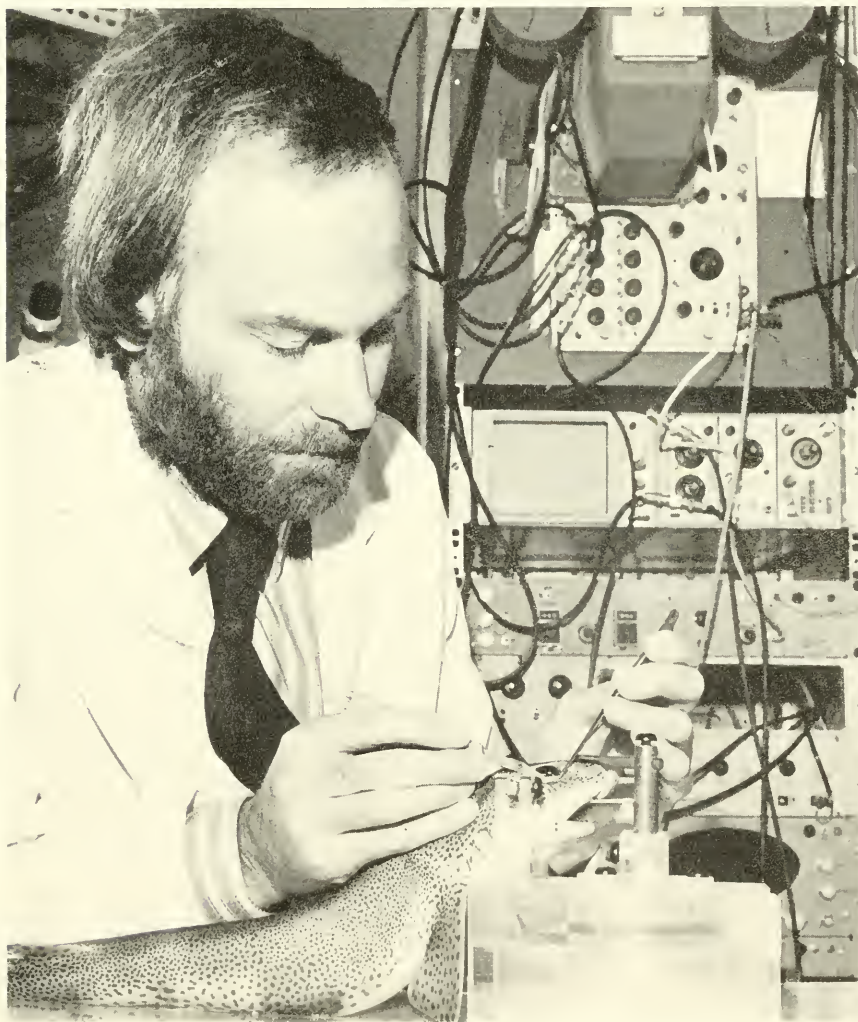
Dr. Barry Roberts prepares for an experiment with an anesthetized dogfish, clamped in a head holder.

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INTRODUCTION

Because most sharks are too large to be kept easily in public aquaria much of our knowledge of their behaviour comes from encounters with sharks in the wild. Although much first-hand information has been acquired in this way, it is seldom objective. These experiences have produced two popular but opposing views about sharks. On the one hand, because of its size, strength, and voracity the shark, more than any other creature, is regarded as an efficient hunting machine. On the other hand, there is the impression that each shark's behaviour is unique and artfully unpredictable. Much of this uncertainty stems from the failure to recognise the environmental features that excite a shark, for what goes on in the brain of a shark, to a large extent at least, is controlled by what is sent into the brain by the sense organs.

Because of the properties of water, mechanoreceptors are an important source of information about the external world for any marine animal.. In pelagic sharks in open water tactile endings of the skin and the vibration sensors of the labyrinth and the lateral line give important environmental information, while the labyrinth and body proprioceptors meet the demands of equilibrium and balance during movement.

This chapter is concerned with summarising current knowledge about these receptors and their importance in elasmobranch behaviour; our first task is to compile a catalogue of the known mechanoreceptors.

A CATALOGUE OF ELASMOBRANCH MECHANORECEPTORS

The sense organs of the skin of fishes, which are used by them to obtain information about the external world, were arranged by Herrick (1903) in three main groups:

1) The taste buds and related chemoreceptors of the *communis system*
2) The *general cutaneous system*, which includes the tactile endings of nerve fibres contained in the spinal nerves of the body and the cranial nerves of the head
3) The *special cutaneous system*, which comprises the sense organs of the lateral line and its derivatives, and which has its own special innervation.

The mechanoreceptors that are the subject of this review are in the last two groups; as well as providing information about the external world, some of them play a significant role in proprioception. The general cutaneous system of elasmobranch fishes contains three types of receptor that may be phylogenetically related, since they are all derivatives of nerve endings. In contrast, the sense organs of the acoustico-lateralis organs are all modifications of ciliated epithelial cells.

The General Cutaneous System

The 'Free' Nerve Ending—The simplest form of mechanoreceptor in fishes, the 'free-ending' termination of sensory nerve fibres, provides tactile,

temperature, and possibly pain sensation and is located near the skin surface. Because the skin of most elasmobranchs contains many hard scales, which are unamenable to conventional histological procedures, most studies on skin innervation have been carried out on whole mounts, stained with methylene blue, or on sections cut through the softer skins of embryos or rays. Such studies (Weddell 1941; Murray 1961) have demonstrated that the cutaneous innervation, which is arranged in the standard vertebrate pattern and consists of two layers of connecting networks, shows little development of specialised endings. In his illustration of part of this plexus, Murray shows myelinated axons (3–6 μm in diameter) of a subdermal network of parallel-running fibres, dividing and losing their myelin before terminating as 'free-endings'. In teleosts such endings measure less than 1 μm in thickness and show no evident distinctions from other small nerve fibres, even at the ultrastructural level (Whitear 1971).

The relative simplicity of this plexus and the skin's resistance to drying provided Murray with a good experimental preparation from which it was possible to record electrical activity by simply placing a wire electrode on the skin surface. In this way he obtained slow, positive-negative impulses that appeared to be initiated some distance from the nerve terminal, into which they then propagated antidromically. The endings were fairly sensitive, giving discharges to movements of less than 20-μm amplitude, but they adapted rapidly to sustained stimuli (75% complete in 5 s). The latency of the spikes (2.2–7.0 ms) depended on the intensity of the stimulus.

The Wunderer Corpuscle—At present only two forms of morphologically specialised nerve terminals have been described in elasmobranch fishes—the *corpuscles of Wunderer*, found in the deeper layers of the skin, and the *endings of Poloumordwinoff*, associated with certain muscle fibres of the paired fins of rays.

The corpuscular endings of the fins of a variety of elasmobranchs, first reported by Wunderer (1908) after whom they are now named, have since been seen in the body by Bone (1964), Tester and Kendall (1967), Roberts (1969b), and Bone and Chubb (1976). The latter authors provide a detailed description of this ending. In the body the corpuscles are nearly always located among the collagenous fibre bundles of the stratum compactum of the dermis, although Tester and Kendall (personal communication) have found some endings deeper, on myotomal septa and close to the vertebral column. Each ending, a whorl of fine unmyelinated fibres, terminates a large axon (Figures 1a, 1b), the myelin sheath of which usually ceases before the ending is formed, although sometimes it penetrates a little way; quite frequently a single axon branches to give a pair of endings.

From their position one might suspect that these end organs would be stimulated not only by touch but also by body and fin movement; this view has been confirmed by Lowenstein (1956). In an electrophysiological study in the pectoral fins of *Scyliorhinus* he demonstrated that these endings responded not only to mechanical pressure but also to fin movement. A study (Roberts 1969b) of the properties of these endings in the body (also in
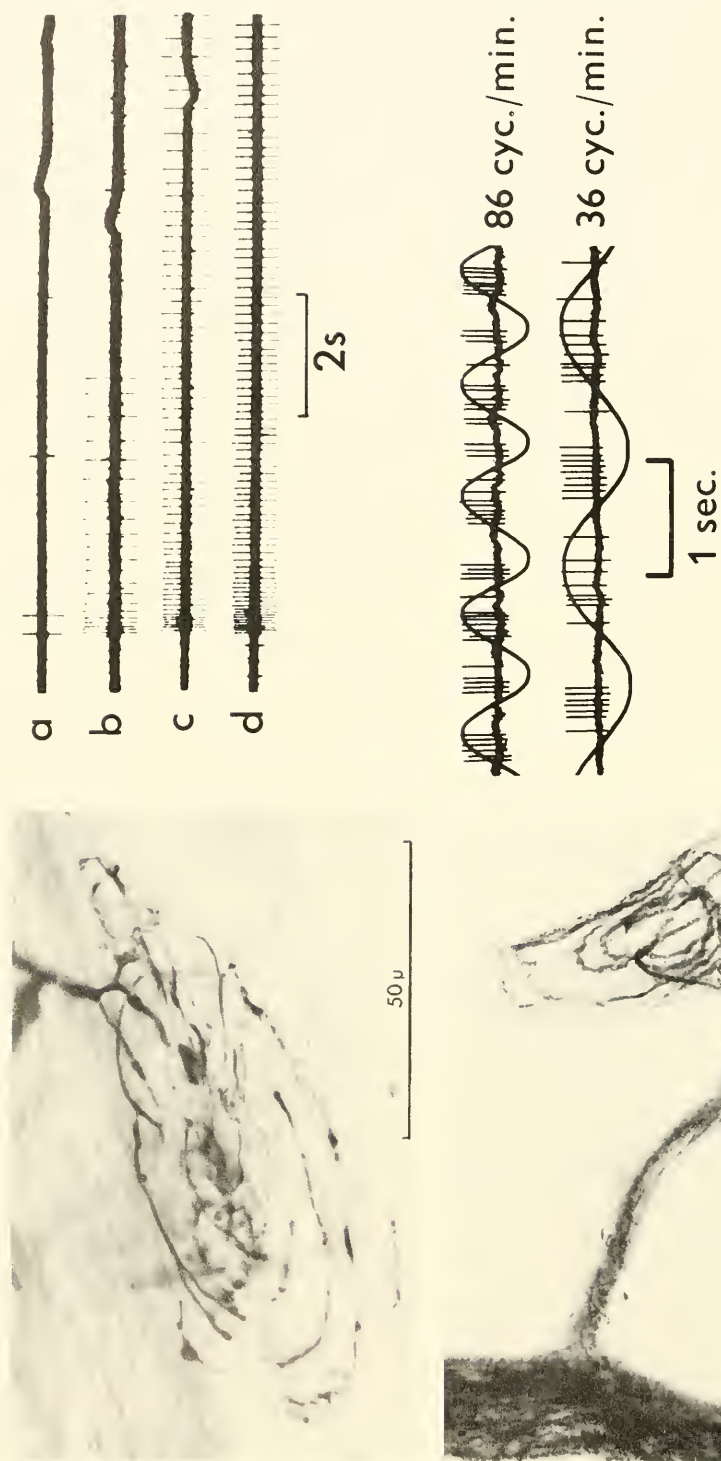
Figure 1 (a, b) Photomicrographs of methylene blue preparations of Wunderer corpuscles in the skin of *Scyliorhinus*. The corpuscle is seen as a whirl of fine fibres, terminating a thick, myelinated nerve fibre. (c, d) The response of a single Wunderer corpuscle to: (c) the body wall being bent through different angles of 36 cycles per minute (a, 5°; b, 10°; c, 15°; d, 20°) and (d) sinusoidal bending at two rates for a constant angle of body bending (20°). (c, d from Roberts 1969*b*).

Scyliorhinus) showed them to discharge to mechanical pressure, skin stretch, and even muscular contraction if this resulted in the skin being pulled, but the natural stimulus appears to be mechanical distortion of the capsule which would be produced by bending movements made by the body during locomotion; i.e., the receptor is to be regarded primarily as a proprioceptor. An attempt to determine the probable behaviour of the corpuscles during locomotion was made (Roberts 1969c) by providing stimuli that simulated body movement. These experiments showed that the receptor was inactive when the body was not bent but that it discharged to a bending movement (Figure 1d), responding to the velocity of the movement and sustaining a slowly adapting tonic discharge, the frequency of which was proportional to the amount of bending (Figure 1c). Apparently the sense organs are not directionally sensitive, as they respond to equal movements of opposite direction.

Mechanoreceptors reported in several elasmobranch electrophysiological studies may be considered Wunderer corpuscles because of their location and discharge properties, although the discharge has not been positively associated with any specific end organ. For example there are endings in shark gills that respond to water flow (Satchell and Way 1962) and to blood pressure (Irving, Solandt and Solandt 1935), and in the jaws are receptors that discharge to pressure on the gums and teeth (Roberts and Witkovsky 1975). Szabo (1962) described a receptor in the caudal fin of *Torpedo* that differs from the usual behaviour of the Wunderer corpuscle in displaying a low spontaneous discharge and in responding to bending of the fin with a discharge of only slightly higher frequency.

The Poloumordwinoff Ending—The intermuscular endings of the fins of *Torpedo*, described by Poloumordwinoff in 1898, have since been found in the pectoral fins of other rays but not as yet in any shark (Cavalié 1902; Barets 1956). These endings consist of many beaded thin nerve fibres that are derived from a single myelinated axon and lie among the muscle fibres of the fins (Figure 2). Unlike the muscle spindle, the simplest form of which is in Urodele amphibia where the nerve fibre swellings are embedded in adjacent muscle fibres, the endings in ray fins are surrounded by Schwann cells that connect only loosely to the muscle fibres; the Poloumordwinoff ending is the simplest vertebrate stretch receptor and may perhaps be regarded as the prototypal muscle spindle (Bone and Chubb 1975).

A brief description of some of the properties of this receptor, in the pelvic fin of *Raja*, has been provided by Fessard and Sand (1937), who reported the presence of a low resting discharge, the increased firing (up to 100 impulses/s) in response to stretch, and the slow adaptation.

In a more recent study Ridge (1977) has confirmed that these receptors are slowly adapting and length sensitive, and he demonstrated that they are also sensitive to the velocity of stretch. The firing frequency of these endings could be affected in various ways by activity of the adjacent muscle fibres and Ridge discusses the possible implications of this in relation to efferent control, which is known to be important in muscle spindle function.

Figure 2 The ending of Poloumordinoff, stained by the method of Winkelmann and Schmitt, situated among muscle fibres of *Raja* pectoral fin (courtesy of Q. Bone).

The obvious structural comparisons between the endings and the muscle spindle raises the fascinating question of whether muscle spindles—specialised receptors arranged in parallel with the musculature—are present in fishes. So far attempts by several histologists, using a variety of stains and material, have failed to reveal spindle-like endings in fish muscle, and the total lack of candidates for a sensory supply has been remarked upon in surveys of fish muscle innervation (Baum 1900; Barets 1961; Bone 1964).

In more recent neurophysiological experiments (Roberts 1969b), proprioceptive discharges were recorded in the spinal nerves in response to muscle stretch and contraction. The discharges did not conform to the recognisable properties of muscle spindles; moreover, they were eliminated by the complete removal of the skin and were therefore attributed to the Wunderer corpuscles. Roberts pointed out that the absence of muscle spindles from fishes may reflect the lack of major postural problems in these animals.

The Special Cutaneous System: The Hair Cell

In the skins of fishes direct sensory contact is made with the external medium by specialised cells that are differentiated from the epithelium and have a sensory role. Chemoreceptors are nearly always of this type, and one specialised mechanoreceptor, the *hair cell*, is also of this form. In the lateral lines of amphibia and some teleosts the hair cell lies in the skin with its sensory hairs protruding into the water, but in elasmobranchs it recedes into a pit, groove, or canal, while in the labyrinths of all vertebrates it is totally withdrawn from the body surface. Although the receptor always has a characteristic form (the hair cell), it is organized with associated structures into an array of sense organs that is sensitive to several sensory modalities; this collection is named the acoustico-lateralis system.

The Structure of the Hair Cell—The hair cell, the associated supporting cells, and the peripheral secretory cells (mantle cells) form a sense organ called the *neuromast*. Each neuromast is surmounted by a gelatinous structure, the motion of which displaces the sensory hairs and thereby (in an unknown manner) activates the hair cells. In some cases this mucilaginous cap extends as a thin sheet called the *cupula*, and the sense organ is then called a *crista*; if the cap is broader and contains a dense calcareous body or bodies (otoliths or otoconia) the organ is called a *macula*. Both types of organ are found in the acoustico-lateralis system.

A typical hair cell, with views of critical areas, is diagrammed in Figure 3. The sensory hairs described by the early morphologists have been shown by the electron microscope to be elongate protrusions, up to 60 per cell. One of these, the kinocilium, is clearly recognisable as a typical cilium because it contains internal tubules of the familiar $9 + 2$ configuration and is attached within the cell by a basal apparatus. The other projections, which are usually shorter than the kinocilium (Figure 4) and are less obviously organized internally, are called stereocilia and enter the hair cell in a specialised region, the cuticular plate. Near the sense organ surface the sidewalls of the hair cell
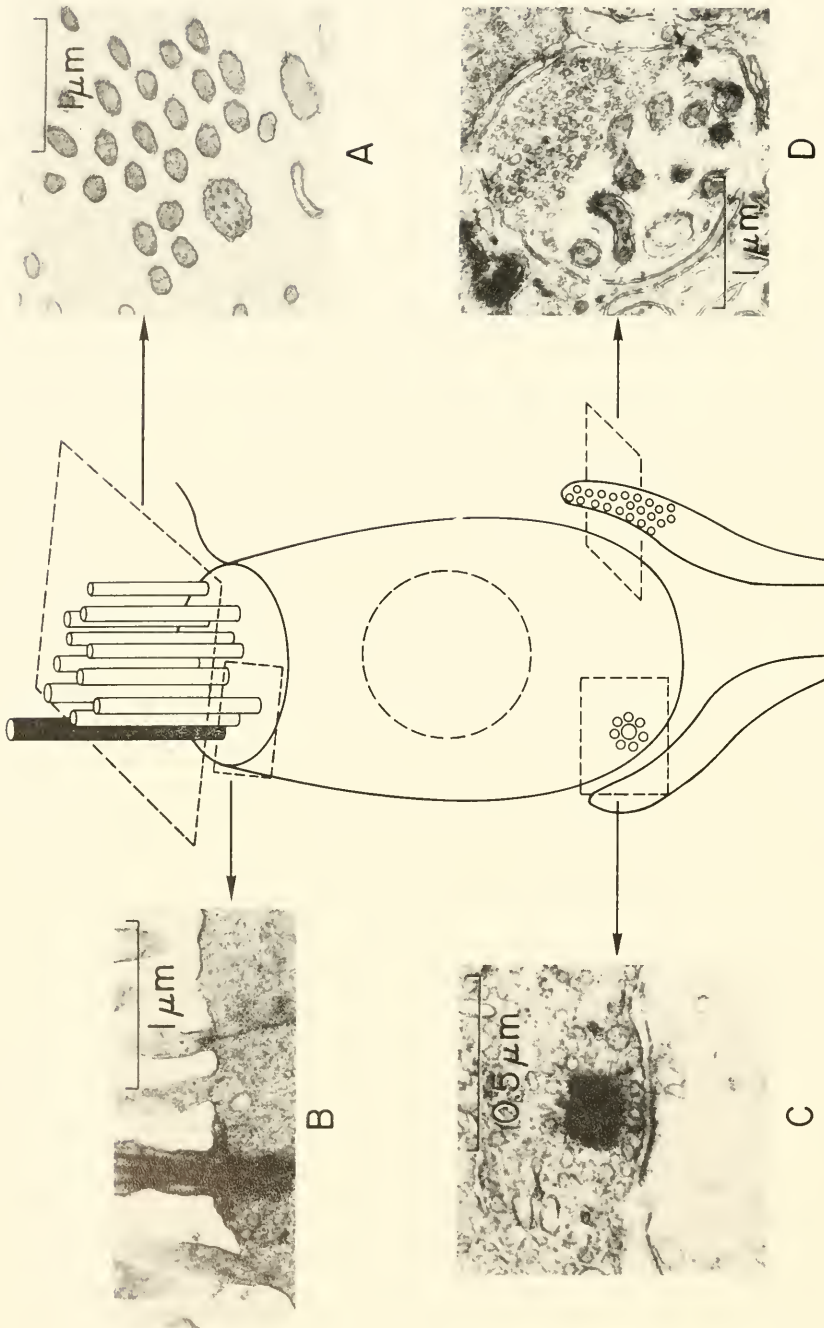
Figure 3 Diagram of the significant features of a hair cell, illustrated with electron micrographs of the lateral-line organ of *Scylio-rhinus*: (A) Transverse section of a hair-cell bundle, showing a group of sterocilia (st) and a kinocilium (k). (B) Longitudinal section through the top of a hair cell, showing the foot (f) of a kinocilium (k) displaying radial striae (rs) just below the cell surface; the axial filaments of a stereocilium (st) unite to form a dense rootlet (r) that penetrates the cuticular plate (cp). (C) Detail of an afferent synaptic body (sb), surrounded by synaptic vesicles (v), is close to the synaptic cleft (arrow), which separates the hair cell (hc) from the afferent nerve fibre (a). (D) Detail of an efferent ending (e); the synaptic vesicles (v) are concentrated toward the hair cell (hc). A cored vesicle is marked by an arrow.

Figure 4 Scanning electron micrographs of sensory hair bundles of the macula neglecta (a) macula lagena (b) of *Squalus*. Note the gradual increase in height of the cilia in a and the very long kinocilium compared with the stereocilia in b. The magnification of a is 10 000 X; that of b is 5300 X. (Photographs courtesy of Dr. D. Bagger-Sjöbäck and Professor J. Wersäll; see also Wersäll and Bagger-Sjöbäck 1974).

are specialised and form desmosomal associations with neighbouring cells, presumably to give the epithelium some mechanical rigidity.

The base of the hair cell makes contact with the lateral-line nerves, which are of two types, one afferent in function and the other efferent. The afferent ending is always present and is associated with clusters of vesicles in the base of the hair cell which are concentrated around an electron dense body called the synaptic body, of which there may be more than one per cell. The efferent ending, which is usually smaller than the afferent terminal, is filled with vesicles; where it forms a synapse there often develops inside the hair cell a closed membranous sac, the subsynaptic cistern. In contrast, in the afferent fibre there is little obvious postsynaptic specialization. So far in fishes, the interesting condition in which the efferent synapse is made on the afferent fibre has been reported only in the neuromast of the goldfish sacculus (Nakajima and Wang 1974).

Most of these standard features can be recognised in those elasmobranch hair cells that have been studied with the electron microscope—in the labyrinth of *Raja* (Lowenstein, Osborne and Wersäll 1964); in the pit organ of *Mustelus* (Hama 1969*b*); in the lateral lines of *Scyliorhinus* (Roberts and Ryan 1971) and *Mustelus* (Hama and Yamada 1977), and in Savi's vesicles of *Torpedo* (Nickel and Fuchs 1974).

The cupula has been demonstrated in shark lateral-line organs by Tester and Kendall (1968), who described it as a delicate structure, poorly preserved in conventional histology but seen clearly in fresh-frozen sections when it fills the lumen of the canal almost completely. The surface is irregular, has no limiting membrane, and stains metachromatically, presumably because it contains mucopolysaccharides. It has a striated appearance and includes cellular debris thought to come from the sensory epithelium. As described by Tester and Kendall the cupula is to be considered a dynamic structure that "grows" at the neuromast surface by incorporating material derived from the supporting cells, while other material is shed distally into the canals, which are kept clear by a slow, head-to-tail endolymph flow. The cupula of *Necturus*, which was visualized with vital dyes and china ink particles, grows at about 15 μm/h (Frishkopf and Oman 1972).

A notable feature of all fish hair-cell systems is that the cilia, although varying in number from fewer than 20 to more than 70, are always arranged in a very characteristic way, with the kinocilium positioned on one side of the ciliary group (Figures 3 and 4). The orientation of the ciliary bundle with respect to its neighbour, and to the axis of the fish, differs in different sense organs. Thus in the teleost lateral line the kinocilia of adjacent hair cells face each other, but in the labyrinth whole clusters of hair cells in the same neuromast have the kinocilium situated on the same side of the ciliary group. In ultrastructural studies on the ray labyrinth, Lowenstein and Wersäll (1959) observed that the kinocilia were on the side of each ciliary group that faced towards the utriculus, in the case of the hair cells of the horizontal canal, but faced away from the utriculus in the crista of the anterior vertical canal. By relating this striking morphological arrangement to the well-established electrophysiological properties of these canal organs (see

later), they developed the interesting hypothesis that a movement of each hair cell bundle towards the kinocilium would excite the hair cell, whereas movement in the opposite direction would be "inhibitory" (Figure 5). This theory has found good support wherever it has been possible to correlate physiological data with morphological observation. For example, in the lateral-line organs some hair cells are excited by one direction of stimulus and some by the opposite direction, and hair cells with kinocilia oriented in both directions have been reported (Flock and Wersäll 1962).
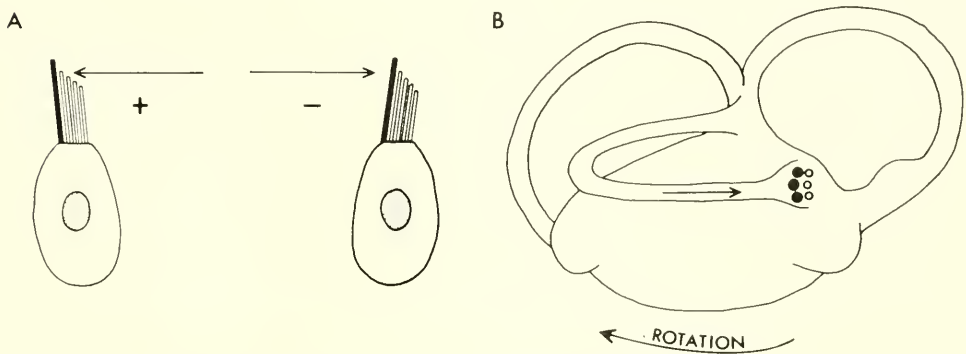
Figure 5   The directional sensitivity of hair cells. Displacement of the ciliary bundle toward the kinocilium (A) excites the hair cell, whereas displacement of the bundle away from the kinocilium decreases hair-cell excitability. In the labyrinth of the ray (B) the kinocilia (shown as "o") are on the outer face of the hair bundle (stereocilia group shown as "●") in the ampulla of the horizontal canal. Rotation as shown causes the endolymph to displace the hair bundle toward the kinocilium and is excitatory (B modified from Lowenstein and Wersäll 1969).

Basic Processes in Hair-Cell Function—As elasmobranch hair cells are not as large as those of amphibia or other species (*Necturus* hair cells are 80 μm by 15 μm—Frishkopf and Oman 1972) and are inconvenient for fine electrophysiology, little detailed work has been done on their function. Nevertheless, because it is probable that all hair cells operate in similar ways, the current ideas on hair-cell function, developed from work on various species, will be briefly summarised here (see also Russell 1976).

It is generally accepted that the adequate stimulus for hair-cell excitation is a shearing movement of the cupula (Trincker 1962), and several theories as to how this actually excites the sense organ have been put forward (Flock 1971; Malcolm 1974; Hillman and Lewis 1971).

An easily recorded extracellular potential, which is set up by a stimulus and is always associated with mechanoreceptive hair cells, is the microphonic potential that can be detected close to the sensory surface (Figure 6), accompanying the stimulus as a negative d.c. potential, but reversing in polarity when the electrode is located beneath the hair-cell epithelium. This potential, which depends on the integrity of the sensory epithelium, is now believed to be a summation of coincident receptor potentials of a large
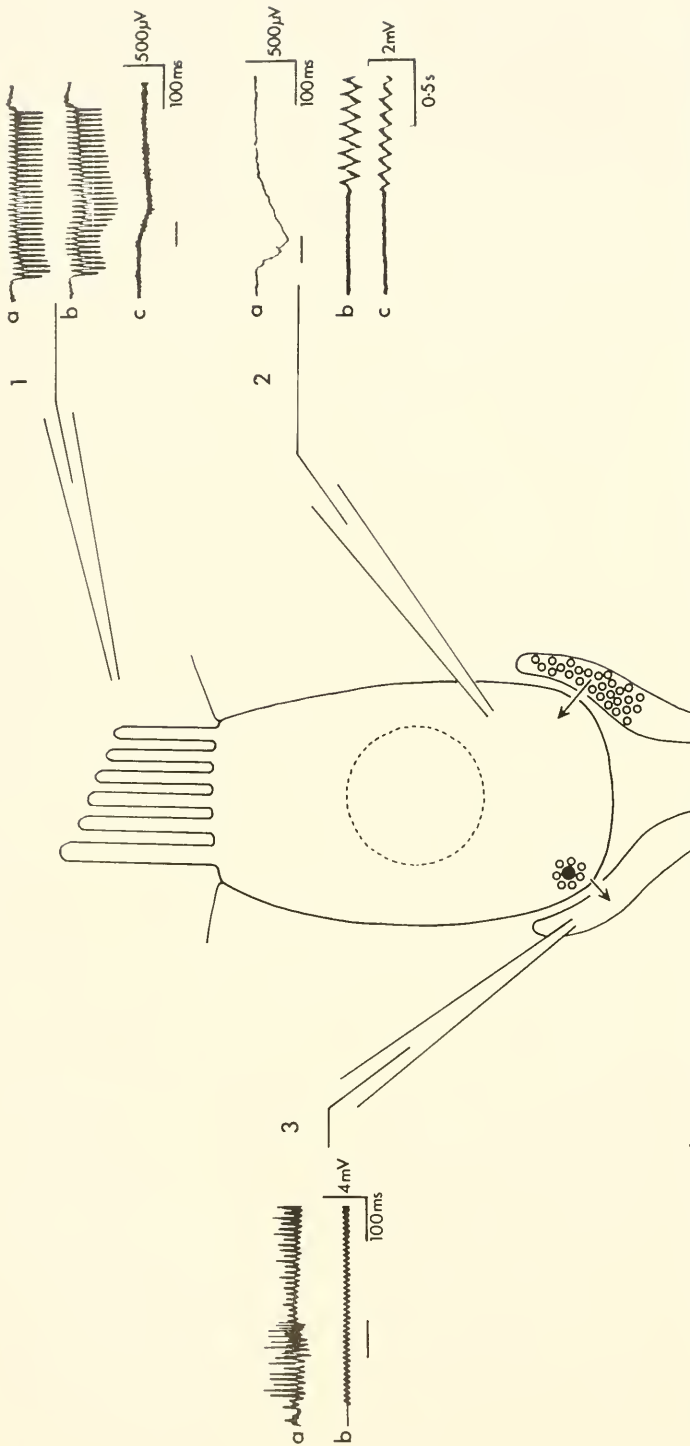
Figure 6   Types of electrical potential that can be recorded in association with a hair cell, illustrated with recordings from the teleost fish *Lota*.   At position 1 the electrode is close to the surface of the hair cell and records the microphonic potential, in response to a 70-Hz vibration of the cupula, which in b increases in size when the efferent nerve is stimulated (horizontal bar below c).   In c is seen the potential recorded during electrical stimulation of the efferent nerve when there is no accompanying stimulation of the cupula.   In position 2 the electrode is inside a hair cell and records (a) an inhibitory postsynaptic potential established by electrical stimulation of the efferent nerve at 200 s^{-1} (marked by horizontal bar).   In b a receptor potential is set up by a 70-Hz mechanical stimulation of the cupula.   In c the electrode has been placed in an adjacent hair cell and records receptor potentials out of phase with those recorded in b.   In position 3 the microelectrode has penetrated an afferent fibre close to the hair cell; excitatory postsynaptic potentials are re-corded during mechanical stimulation of the cupula.   These are attenuated after the efferent nerves have been stimulated (at 200 s^{-1}, for duration of horizontal bar).   Trace b shows the 70-Hz vibration.

number of hair cells. Like that of other sensory receptor potentials, the amplitude of the microphonic potential is linearly related (on a semilog scale) to the amplitude of the stimulus (e.g. Furukawa, Ishii, and Matsuura 1972a).

Because of its ease of detection many studies have been made with the microphonic potential. For example, in bony fishes the recorded microphonic has provided data on hearing thresholds, directional sensitivity, and the audiogram range. Microphonics have also been recorded from lateral-line organs where, interestingly, they occur at twice the frequency of the applied stimulus (Jielof, Spoor, and de Vries 1952; Flock 1965), an explanation for which was provided by Flock on the basis of the polarity of the hair cells as seen with the electron microscope (Flock and Wersäll 1962); the double responses recorded from the goldfish sacculus (Furukawa and Ishii 1967a) and from near the shark macula neglecta (Fay, Kendall, Popper, and Tester 1974) presumably have a similar basis. The only published record of microphonics from an elasmobranch receptor is that given by Lowenstein and Roberts (1951) although data from microphonic recordings were used by Fay et al. (1974).

It is worth emphasizing that if the microphonic does represent the receptor potential of the hair cells it may provide data on hair-cell function but it cannot be used to provide reliable data on the range or the threshold of the *sensation* of hearing, because there is no certainty that the hair cell afferent-fibre synapse will respond in a similar way to the microphonic nor, indeed, that the higher sensory centres will not modify this signal.

Receptor potentials—Intracellular recordings have now been successfully made for several different lateral-line organs: hair cells of *Necturus* (Harris, Frishkopf and Flock 1970; Yanagisawa, Taglietti, and Katsuki 1974; Sand, Ozawa, and Hagiwara 1975); *Lota* (Flock and Russell 1973a, b), alligator-lizard ear (Weiss, Mulroy and Altmann 1974) and mammalian cochlea (Russell and Sellick 1977). In these examples very sharp, dye-filled electrodes were used so that the location of the electrode tip could be confirmed by dye staining. The resting potentials of the hair cells appear to be rather low (42 ± 13 mV (Yanagiswa et al. 1974); 33 ± 13 mV (Sand et al. 1975)) except for Szabo and Hagiwara's (1966) values of 40–70 mV for the epithelial cells of Savi's vesicles in *Torpedo*, but the exact recording site was not identified precisely. Intracellular recording in *Necturus* has also shown that the resting potential level can be biased by sustained displacement of the cupula, the effect being to depolarize or hyperpolarize the cell, according to the direction of stimulation (Flock 1971).

In their first recording, Harris et al. (1970) used computer averaging techniques during applied repetitive stimulation and observed small oscillating changes that did not exceed 800 μV in size. Such potentials (Figure 6b) would certainly be too small to function as generator potentials in nerve fibres, but it is possible that the hair-cell/afferent synapse is a sensitive amplifier that responds to such small potentials. Recently Sand et al. (1975) found that afferent nerve firing could be evoked in hair cells stimulated by

small currents that established potentials comparable in size to these receptor potentials. They also found, in contrast, that no change in the afferent fibre discharge could be brought about by currents even 10 times as large that were injected directly into the afferent fibre.

In the lizard ear essentially similar potentials have also been recorded in supporting cells, and on this basis Weiss et al. (1974) suggest that the supporting cells and the hair cells might be coupled electrotonically. Such coupling would not be totally unexpected, for Hama (1969a) has already reported the presence of "gap junctions"—the accepted morphological basis for electrotonic coupling—between neuromast cells in the fish *macula sacculi.*

Postsynaptic responses—The changes in the hair-cell resting potential, seen as the receptor potential, presumably lead in some way to the release of the transmitter at the hair-cell/afferent-nerve synapse, for intrafibre records close to the hair cell have provided confirmatory evidence that these synapses function in the manner now well understood for chemical synapses. Thus, spontaneous, small-amplitude, erratic potentials—supposedly miniature potentials—have been seen in the afferent terminals that make connection with Savi's vesicle (Szabo and Hagiwara 1966), the lateral-line (Flock 1971), and saccular hair cells (Furukawa and Ishii 1967b; Furukawa, Ishii, and Matsuura 1972b). Mechanical stimulation promotes a massive increase in the size of these potentials (Figure 6) which, on reaching a certain level, initiate spike firing. These authors also report (Ishii, Matsuura, and Furukawa 1971) that the amplitudes of the microphonic potentials and of the excitatory postsynaptic potentials (EPSP's) are related in a sigmoid fashion; these potentials are functioning therefore as generator potentials, for increases in their size lead to enhanced firing of the afferent nerve fibres. These postsynaptic potentials follow the microphonic with a delay of only 0.6–0.8 ms.

In all cases so far studied the influence of the efferent innervation on the hair cells has been found to be inhibitory, causing a decline or abolition of afferent activity. The cellular basis of this inhibition has now been unravelled by means of intracellular measurements from the hair cells and concurrent recordings of microphonic potentials. Flock and Russell (1973a, 1976) have successfully recorded from the lateral-line hair cells of the teleost *Lota* and found that a train of stimuli applied to the efferent nerve results in the development of a hyperpolarizing potential in the hair cell, up to 10 mV in amplitude, which outlasts the stimulus by 150–200 ms (Figure 6). This hyperpolarization of the cell should effect a decrease in transmitter release, and recordings from afferent terminals during efferent-nerve stimulation have shown a reduction in the EPSP's established by mechanical stimulation (Figure 6).

The inhibitory impact of the efferent terminals is registered by an extracellular electrode placed close to the sense organ in two ways (Flock and Russell 1973b). First, as would be expected for an electrode sited in a volume conductor some distance from an inhibitory synapse, a negative

potential (Figure 6) that has the same time course as the IPSP is recorded. Second, if the sense organ is displaced mechanically at the same time as the efferent nerves are stimulated, the microphonic potential becomes larger (Figure 6). This is because efferent nerve stimulation causes a small increase in hair-cell membrane conductance.

Transmitter substances—Until quite recently it was acceptable to suggest that the microphonic potential directly excited the nerve terminals, but now all the electrical measurements, when taken together with the ultrastructural data, indicate that the hair cells act on the afferent fibres via chemical synapses. The electron microscope shows that both the hair cell and the efferent ending contain many vesicles. In the efferent terminal these are usually of two morphological types: a single population of lightly staining vesicles of spherical form, with a mean diameter of around 60 nm, and a few larger vesicles with a densely staining core. The vesicles that cluster around the hair-cell synaptic body constitute a single population with a mean size of about 50 nm.

The least confusing pharmacological data on the nature of the transmitter substances are for the efferent endings, which are thought to be cholinergic (Russell 1971*b*). The story for the afferent terminals is much less clear. Recent studies by Flock and Lam (1974) on the bullfrog basilar papilla, which has only an afferent innervation, showed that GABA was synthesised in the neuromast, while in the lateral-line organ of the toadfish and the ampulla of the skate (*Raja*), where both types of terminal are present, both GABA and acetycholine were synthesised. On this basis, therefore, Flock and Lam argued for GABA as a transmitter candidate at the afferent terminal, and they went on to show that picrotoxin, which specifically blocks GABA-transmitting synapses, stopped both spontaneous and evoked activity. However since then, Sand et al. (1975) have applied picrotoxin and bicuculline to the hair cells of *Necturus* and found no change in sense-organ sensitivity.

Other substances have been suggested as the transmitter at afferent synapses. On the basis of the changed appearance of the afferent synaptic body in the frog labyrinth after treatment with reserpine and guanethidine, Osborne and Thornhill (1972) suggested that catecholamines must be present at afferent terminals, while Steinbach and Bennett (1971) found that glutamate had an excitatory action on the related hair cells of the ampullae of Lorenzini. Clearly, the nature of the afferent transmitter substance remains a problem.

Construction of Hair-Cell Organs: The Acoustico-Lateralis System

In nearly all cases the hair cell functions as a directionally sensitive displacement detector. The "free" neuromasts found in some teleosts and aquatic amphibia respond to water velocity (Harris and Milne 1966), but in the canal system, where the sense organs are connected with the outside world by special pore openings, the neuromasts register water displacement. Fluid displacements set up by angular acceleration of the head are the primary

stimulus for the semicircular canals, while in both the utricular and saccular neuromasts the cilia are embedded in a gelatinous mass that is loaded with calcium carbonate particles that render the sense organ sensitive to linear accelerations (including gravity). The modality of the sensory systems built around the hair cells depends on the relationship between the sense organs and the ancillary structures.

Free Neuromasts: the "Pit Organs"—Scattered over the head and body of all elasmobranchs are neuromasts that project into the outside world rather than into canals. These free neuromasts lie in slits in the skin of rays and primitive sharks (e.g. *Heptanchus*) and in pits between specifically modified scales in sharks. A description of these organs in various elasmobranchs is provided by Johnson (1917), Budker (1958), and Tester and Nelson (1967), where the modified scales and patterns of pit organ distribution are particularly well described.

The sense organs in each pit form a round (e.g. *Sphyrna*) or elongate (e.g. *Squalus*) domed structure, 100–200 μm diameter, which is set among the modified scales. The dome is covered by a mucilaginous plug, but Tester and Nelson (1967) were uncertain whether this was really a cupula, as the usual striations are absent. Budker (1958) was impressed by the apparent similarity in form between the pit organ and the teleostean taste bud and postulated a chemoreceptive function for the pit organs. He thought he had evidence when he found behavioural responses to food extracts applied directly to pit organs. However, in Tester and Nelson's view the pit organ closely resembles the lateral-line neuromast, an observation supported by the electron microscopic description of *Mustelus* pit organs, where the sensory cells are seen as hair cells with stereocilia and kinocilia (Hama 1969*b*).

So far the only neurophysiological attempt to determine pit organ function has been that of Katsuki, Yanagisawa, Tester, and Kendall (1969) in *Ginglymostoma*; recordings were taken from filaments of the posterior lateral-line nerve while mechanical and chemical stimuli were applied to the caudal fin receptors. Although both canal and pit organs are innervated by the same nerve bundle, their responses could be distinguished; for although both were spontaneously active, the canal organ had a much lower threshold to mechanical stimulation. In addition, the pit organ stopped discharging when it was exposed to distilled water, responded vigorously to monovalent cations (e.g., 1 M NaCl) and was inhibited by calcium and magnesium; anions and sugar however were ineffective. It was also found (Katsuki and Hashimoto 1969) that monovalent cations enhanced the mechanosensitivity of these sense organs.

Canal Organs—The neuromasts of the canal system are housed in a series of canals distributed over the head and along each side of the trunk in a pattern that forms a conspicuous feature of fish anatomy. The history of their discovery is well described by Ewart (1892), who reviews the earlier literature. The system appears to have been recognised first, in the elasmobranchs, as openings discharging mucus (by Stenonis in 1664). Lorenzini

(1678) subsequently distinguished between the canal system and the gelatinous tubules bearing the ampullae now named after him, but the whole system was regarded as mucus-secreting until in the late nineteenth century Leydig and Schultze put forward the idea that it was really a sensory structure, perhaps serving a "sixth sense."

The pattern of the canals—Although the distribution of the canals is discernible in some species on superficial examination, for most the canals have to be exposed in some way, either by injecting dyes or by serial sectioning of embryonic material.

The nomenclature of the various canals has caused much confusion and was subject to considerable variation until Ewart (1892) appreciated that the distribution of the canals is strongly influenced by their innervation. The scheme which he then elaborated has, for the most part, been followed by subsequent writers. Accordingly, the canals constitute the following (Figure 7A);

1) the *lateral canal*, innervated from the lateralis division of the vagus nerve, and running from the tip of the tail to the head, where just behind the orbit, it divides into:

2) the *supraorbital canal*, innervated by superficial ophthalmic VII, which passes over the eye and

3) the *infraorbital canal*, innervated by buccal VII, which runs under the eye, off which branches

4) the *hyomandibular canal*, innervated by external mandibular VII. A short canal, the *supratemporal*, crosses the head to unite the two lateral canals, while discrete *mandibular canals* lie on each side of the lower jaw.

In some way the configuration of these canals presumably reflects hydrodynamic flow over the head and body, but its biological significance remains a mystery.

Apart from Ewart's original description of *Somniosus*, full accounts of the form of the canal system in a great variety of elasmobranchs are provided by Garman (1888), Ewart and Mitchell (1892), Johnson (1917), Norris (1932), and Von Bonde (1933a, b).

Some canals of *Chlamydoselachus* (Hawkes 1906) and of *Chimaera* (Cole 1896) consist merely of a groove set into the surface of the skin. In other forms these grooves are closed for at least part of their length, while in most elasmobranchs these tubes then lie beneath the epidermis and connect to the outside by means of tubules, which Ewart thought of as 'feelers'. In *Chlamydoselachus*, however, some canals lack tubules and open directly. The tubules are mostly quite short, but in some species they may be very long and branching, as on the tail of the thresher, *Alopias*. In front of the eye the tubules generally run anteriorly, whereas behind the eye the pores usually face the tail.

Although the canal system of most species conforms to the general pattern just described, variations are found (Figure 7). For example the
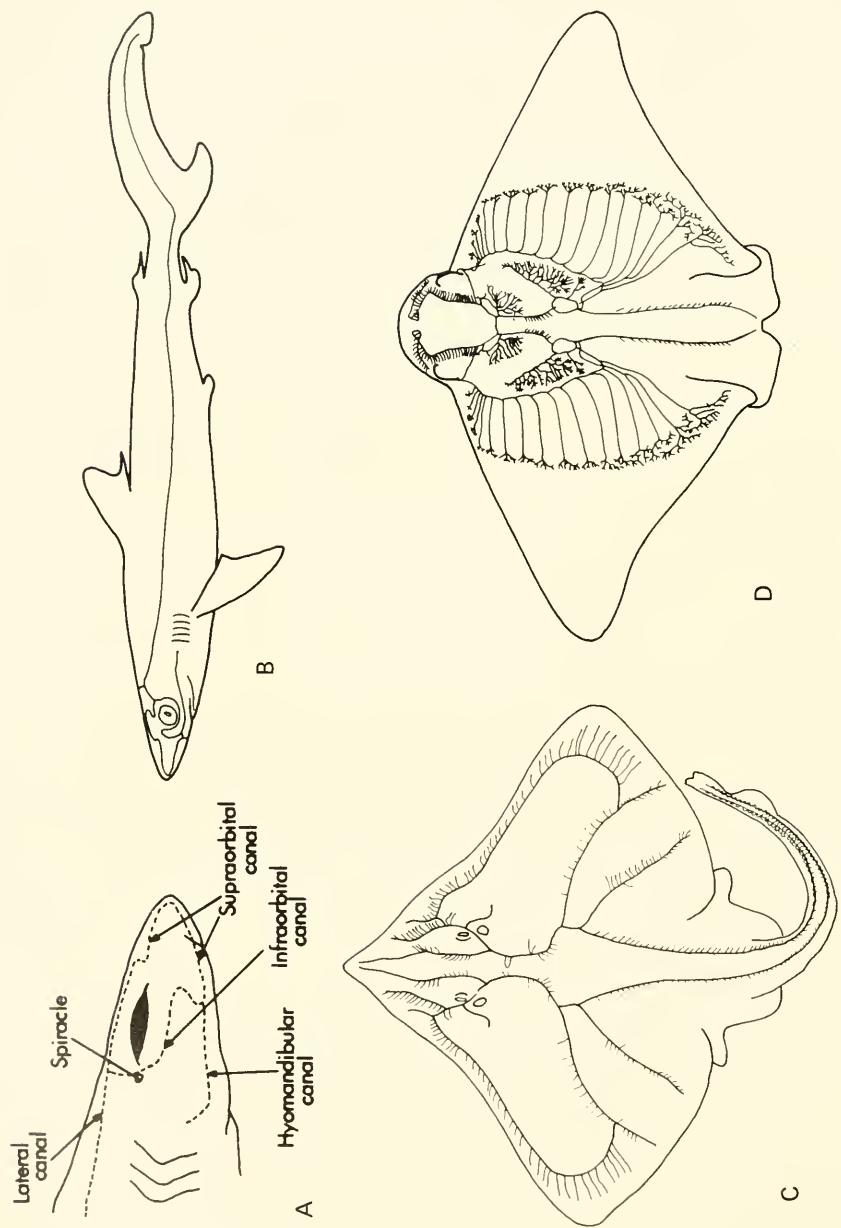
Figure 7 The organization of the lateral-line canal system in some elasmobranchs: (A) the head of *Scyliorhinus* (side view); (B) *Carcharhinus* (side view); (C) *Raja laevis* (dorsal aspect); (D) *Myliobatis* (dorsal aspect) (redrawn from Tester and Kendall 1969*b* and Gudger 1888*c, d*).

arrangement in rays differs considerably from that of sharks, particularly in the length of the hyomandibular canal, although most writers would accept Garman's view that the batoid configuration could be transformed from the shark arrangement, if the starting form was a fish like *Chlamydoselachus*. In other species, like *Pristiurus* with its elongate snout or *Sphyrna* with its bizarre head, the normal pattern is of course distorted, while in bottom-dwelling species like *Raja* the ventral canals have few openings (Figure 7C) or are absent (*Torpedo*); these canals are well formed and have extensively dichotomizing tubules in open-ocean rays such as *Myliobatis* (Figure 7D).

Histology of the canal system—Although tubules and canals are both lined with two-layered epithelium, only in the canal, where the dorsal wall is modified to form an almost continuous sensory epithelium, are neuromasts to be found (Figure 8).
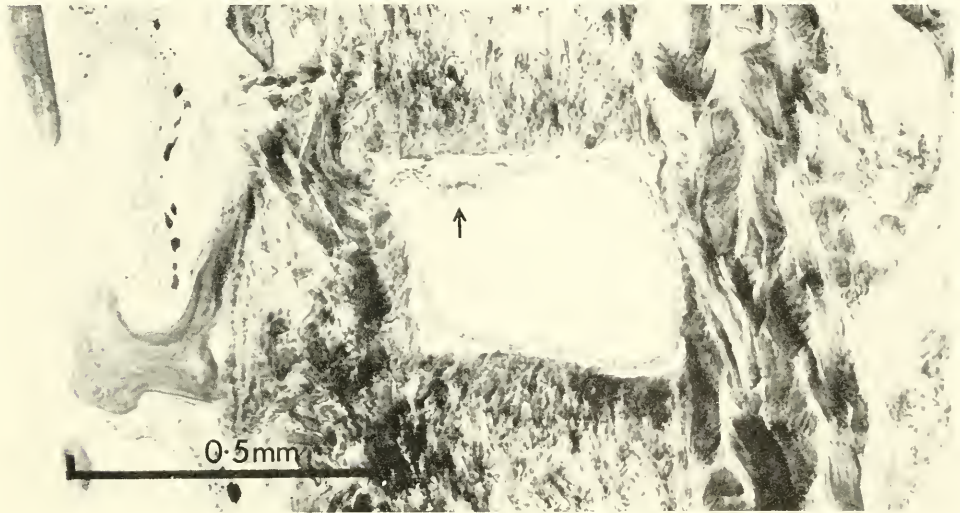
Figure 8   Light micrograph of a transverse section through the body wall of *Scyliorhinus*, showing the lateral-line canal lined with an epithelium that is swollen at the neuromast (arrow).

The centre of each neuromast, decked by its own cupula, lies opposite an opening of a tubule so that, except where this branches, the number of neuromasts is equal to the number of pores. This number differs among species even for the same canal; thus the infraorbital canal of *Mustelus* contains 110 organs (Allis 1901) but that of *Chlamydoselachus* only 52 (Hawkes 1906). The organization of the canal epithelium has been well described at the level of the light microscope for *Mustelus* by Johnson (1917) and for *Carcharhinus* by Tester and Kendall (1969), while the ultrastructure of the cells is outlined by Roberts and Ryan (1971) and Hama and Yamada (1977).

The canals of the lateral-line system have generally been assumed to contain either mucus (they were, it will be recalled, first thought of as a mucus-secreting system) or a special fluid as found in the labyrinth, where the hair cells are bathed in a viscous liquid that is particularly rich in potassium and low in sodium when compared with tissue fluid.

Values obtained in recent analyses, given in Table 1, show that the ionic contents of the lateral-line fluids are different from ear endolymph. In *Scyliorhinus*, for example, where the canal system is open to the sea, the endolymph is indistinguishable from seawater, but in fish in which the canal system is closed, such as *Corphaenoides* and *Lota*, the endolymph potassium is of similar concentration to, or even more concentrated than, the tissue

Table 1.   Ionic contents of various endolymphs and seawater (mM/ℓ)

	K⁺	Na⁺	K⁺/Na⁺	Source
Seawater	11.7	536	0.02	Liddicoat and Roberts 1972
Lateral line				
Scyliorhinus	12.0	509	0.02	
Raja (in Baltic sea)	7.7	287	0.02	Ilinsky and Krasnikova 1971
Coryphaenoides	2.6	169	0.02	Fänge, Larsson, and Lidman
Lota	27.1	120	0.23	1972
Xenopus (cupula)	24-100	—	—	Russell and Sellick (1976)
Labyrinth				
Squalus	60.0	280	0.21	Vilstrup and Jensen 1964
Raja	63.4	295	0.22	Murray and Potts 1961
Chrysemys (turtle)	114	2.7	42.2	Johnstone, Schmidt, and Johnstone 1963
Rattus (rat)	154	0.9	171	Bosher and Warren 1968

The K^+/Na^+ notation is used in the header for potassium-sodium ratio.

fluid potassium. The open canal system can probably be regarded therefore as the primitive condition, and changes in the endolymph composition became possible only when the canals were closed. Even in systems in which the neuromasts are exposed to a low K^+ environment, a high K^+ microenvironment appears to exist within the cupula, immediately adjacent to the hair cell surface (Russell and Sellick 1976).

Neurophysiology of lateral-line organs—The properties of the lateral-line organ have been examined in a number of fish preparations by recording from single filaments teased from lateral-line nerve bundles. Hoagland (1933) carried out this type of study on teleosts and was the first to show that the unstimulated lateral-line organ is spontaneously active, an important observation that has since been frequently confirmed. He was uncertain about the significance of this resting discharge, but shortly afterwards a similar feature was recorded by Lowenstein and Sand (1936) in the elasmobranch labyrinth and was shown by them to provide the basis for directional responsiveness.

Statistical analyses of lateral-line spontaneous activity have been carried out for *Xenopus* (Harris and Milne 1966) and for the eel *Anguilla* (Alnaes 1973*a*), and some data on the dogfish *Scyliorhinus* are given by Roberts (1972). In the dogfish some units discharge regularly, and nearly all the interspike intervals fall within 40 ms of each other (Figure 9b); others show greater variation, which is more marked after a period of strong mechanical stimulation. Alnaes (1973*a*) has pointed out that regular firing probably means that there is only a single spike initiation site; in *Xenopus*, with irregular firing, evidence for multiple initiation sites has recently been presented (Murray and Capranica 1973).
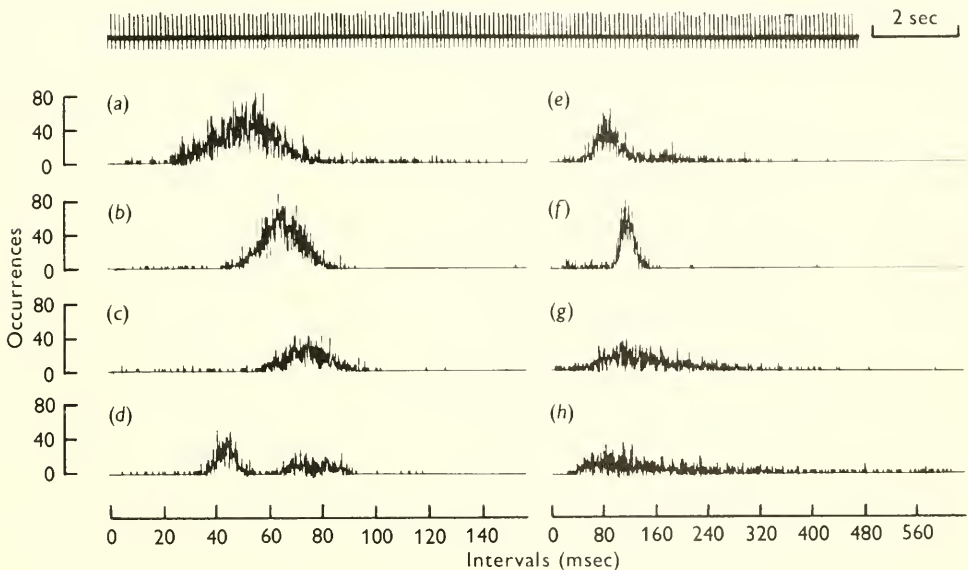
Figure 9   Typical record of a spontaneously discharging lateral-line unit from *Scyliorhinus*. Histograms of 1000 consecutive impulses from eight different units illustrate the various patterns of spontaneous activity found in the posterior lateral-line organs (Roberts 1972).

Although it is widely agreed that the stimulus for the hair cell is a shearing displacement of the cupula, how this arises naturally is a question causing much controversy which we shall be trying to answer more fully later. Sand (1937) argued that the consequence of the stimulus, however generated, would be a movement of the fluid in the canals. He compared the sensory hairs to "reeds in a river bed . . . bent while the stream flows"—and so in his established certain important features about lateral-line organization. First, he found, in opposition to previous views, that the sense organ adapted slowly to a sustained stimulus. Second, he showed that some units were excited if the fluid flowed in one direction along the canal but were depressed (he spoke of "inhibition") if the flow went the other way (Figure 10).
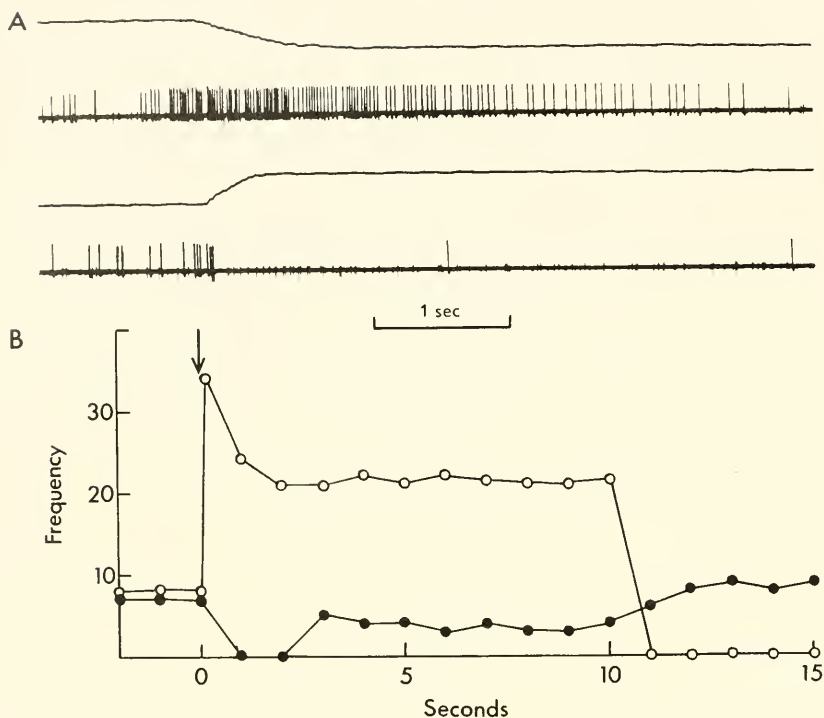
Figure 10 Directional responsiveness of lateral-line organs. (A) A single efferent fibre in *Scyliorhinus*, in this case responding to body movements, showing an accelerated discharge to one direction of movement (indicated by the upper trace of each record) and a cessation of activity to a movement of opposite direction (Roberts 1972). (B) Impulse frequency (s^{-1}) of a unit in *Raja* responding to the flow of Ringer solution along the canal (beginning at arrow and lasting for 10 s. In one direction the flow is excitatory (o); in the opposite direction it is inhibitory (●) (redrawn from Sand 1937).

Savi's Vesicles—These organs, named after their discoverer, are vesicular structures 2–3 mm in diameter, found in rows on the dorsal and ventral surface of the anterior edge of the disc of electric rays. Coggy (1891) and Norris (1932) established a good case for believing that these organs are vestigial remnants of the ventral canal system which otherwise is absent from these fishes; more recent studies on structure and neurophysiology support this interpretation (Szabo 1968). Each vesicle consists of a sensory surface containing three neuromasts, totally covered by a cupula; at the ultrastructural level these neuromasts all seem to consist of slender hair cells (with dual innervation) and adjacent supporting cells (Derbin and Szabo 1966; Nickel and Fuchs 1974).

Fessard and Szabo (1958) (and Szabo and Fessard 1965) have made recordings from the afferent supply of these receptors and found tonically discharging units that responded to stimulation by an increase or decrease of

the basic discharge, depending on the direction of the stimulus, and other units that responded only on application of the stimulus.

The Labyrinth—Because of the transparency of the cartilaginous skull, and because of its size, the elasmobranch labyrinth has provided significant anatomical and experimental material for the study of labyrinth function in general. This organ has the same basic form in all vertebrates (Figure 11) and consists of two large sacs, the sacculus and utriculus, off which open the semicircular canals. Each canal, of which there are three in selachians, extends from the ultriculus in an arc and bears at one end a swelling (the ampulla) which houses a cupula-bearing sense organ.

DUCTUS ENDOLYMPHATICUS

POSTERIOR VERTICAL CANAL

ANTERIOR VERTICAL CANAL

HORIZONTAL CANAL

MACULA NEGLECTA

LAGENA

CRISTAE

UTRICULUS

SACCULUS
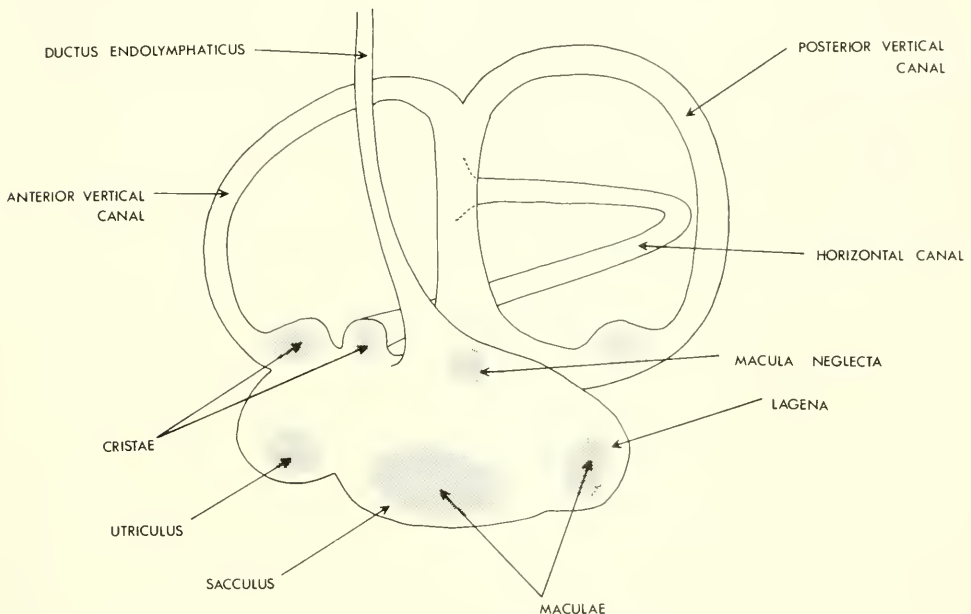
MACULAE

Figure 11   Simplified diagram of the elasmobranch labyrinth.

The thin-walled utriculus houses the macula utriculi, a neuromast covered by a mucilaginous cupula in which many calcium carbonate granules (the otoconia) are embedded (mean size = 10 μm (Carlström 1963)). The sacculus, a triangular sac, also contains an otoconia-bearing sense organ, the macula sacculi, and expands at one end with a protrusion, the lagena, which houses another neuromast. Another sense organ, the macula neglecta, though it seems to lack an otolith (Tester, Kendall, and Millisen 1972), is near the junction between the posterior vertical canal and the sacculus. Dorsally the sacculus opens into a narrow tube, the endolymphatic duct, which passes through the chondrocranium to open to the seawater. The canal lumen contains granules of calcium carbonate (sometimes sand) of the same type and size as the otoconia (Carlström 1963). In the roof of the skull at this

point is a depression, the parietal fossa, which is filled with connective tissue and on the floor of which is an opening, the fenestra ovalis, closed on the side of the fossa by a membrane and, within the skull, by the wall of the posterior vertical canal. This specialised arrangement suggested to Tester et al. (1972) a role in sound detection, in which the endolymphatic duct would function in pressure equalisation.

Numerous accounts of the form of the easily dissected elasmobranch labyrinth are available in the literature, beginning with the excellent descriptions by Retzius (1881). Stewart (1905, 1906) characterized eight species not illustrated by Retzius, and Werner (1930) reviews all the literature and figures 21 species selected from the main elasmobranch families. Additional details are found for *Squalus* in Vilstrup's monographs (Vilstrup 1950, 1951) and for *Carcharhinus* in Tester et al. (1972). Good descriptions of development are provided for *Mustelus* by Ayers (1892) and, especially, by Quiring (1930) for *Squalus*.

From his review Werner (1930) appreciated that the elasmobranch labyrinth is found in two basic forms. In one, as in *Carcharias*, there is a small sacculus, a long endolymphatic duct, and a *crus commune* formed from adjoining vertical canals (Figure 12B); in the other, there is a large sacculus, and although the anterior vertical and horizontal canals share the same portion of the utriculus, the posterior vertical canal is separate. This form is much more common and is illustrated by *Lamna* (Figure 12A).
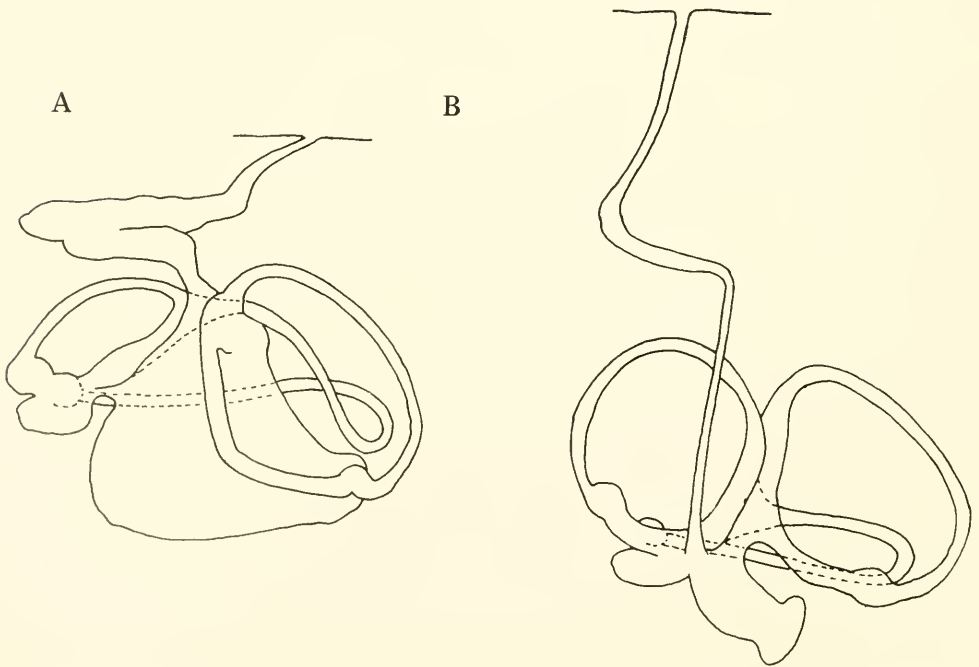
Figure 12 Two examples of elasmobranch labyrinths seen from the medial view, with the anterior vertical canal on the left (A) *Lamna*: (B) *Carcharias* (redrawn from Stewart 1905).

The form of the canals and their function—The important studies of Steinhausen (1933) on the labyrinth of the pike showed that during rotatory acceleration of the head the endolymph and cupula were rigidly coupled. This lead him to develop the torsion-pendulum model of semicircular canal function.

Following Steinhausen, the general view has developed that the semicircular canals are responsive to angular accelerations and that linear accelerations are registered by the other sense organs. Much evidence has been accumulated that supports this view, although most recently it has been shown that in some animals, including elasmobranchs (Lowenstein 1974), the canals also respond to linear accelerations, presumably because slight differences in density exist between the cupula and the endolymph (Goldberg and Fernandez 1975).

Considerable insight into semicircular canal function was obtained from elasmobranch preparations, in which the size, accessibility, and arrangement of the nerve bundles permitted unit records to be taken from the afferent fibres. The experiments, carried out on the horizontal canal of the dogfish and on all three canals of the ray (Lowenstein and Sand 1936, 1940*a,b*), established that at rest most units showed a steady discharge, which increased during constant ipsilateral acceleration and decreased or stopped during constant contralateral acceleration in the canal's plane (Figure 13B).
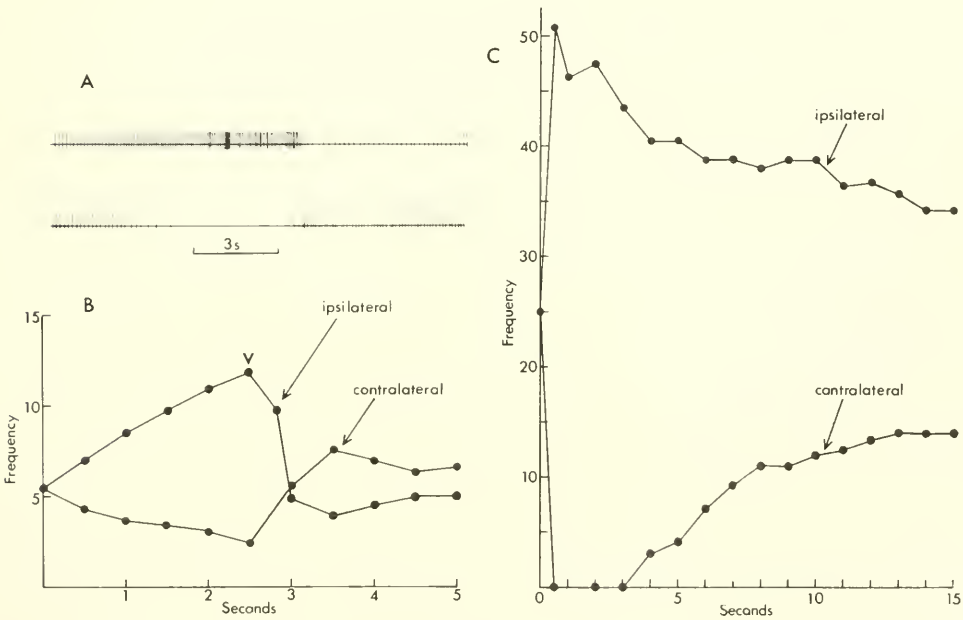
Figure 13 Directional responsiveness of horizontal semicircular canal and organ in *Raja*. (A) Tracing of single end organ discharge in response to continuous angular acceleration. (B) Changes in impulse frequency (s^{-1}) during ipsilateral and contralateral rotation (stopping at arrow). (C) Change in impulse frequency during ipsilateral and contralateral rotation at constant angular velocity (redrawn from Lowenstein and Sand 1940*a*).

With rotation at constant velocity (Figure 13C) there was an initial abrupt increase or decrease in discharge rate, followed by a slow return to the resting frequency. Lowenstein and Sand (1940a) found a threshold effect somewhere around $3°/s^2$, which ten Kate (1973) calculates as being a deviation of the hair cell cilia of about 0.1 nm.

The experiments also showed that in *Scyliorhinus* the horizontal canal responded only to horizontal rotation, whereas the vertical canals could register rotation in all three axes. These out-of-plane responses are much less pronounced in *Raja* (Lowenstein 1974). O'Leary and his collaborators (O'Leary, Dunn, and Honrubia 1974; O'Leary and Honrubia 1976), working with the horizontal canal of *Rhinobatus*, suggested that the sense organs receive a systematic projection of nerve fibres in individual bundles that behave quantitatively differently from each other when exposed to rotations. From this finding they developed a theory that each afferent fibre is 'tuned' to a particular range of head accelerations.

Groen, Lowenstein, and Vandrik (1952) used the elasmobranch canal system to examine the torsion-pendulum theory closely by recording from horizontal canal afferent fibres of *Raja* during sinusoidal movement and in response to sudden changes in the velocity of a rotating turntable. They confirmed the presence of a resting discharge of 6–100 imp/s (mean 26.5), which they found to be very constant, deviating by only about 4% from the mean, and went on to show that during a sinusoidal movement the time the impulse frequency was at the "resting" value did not coincide with zero position (i.e., there was a phase difference) and that it took at least 55–100 ms for the impulse discharge to return to its resting value after a sudden acceleration. They assumed that this long delay was a reflection of the slow return of the displaced cupula to its resting position.

Groen et al. (1952) believed that their data conformed satisfactorily to the equations that describe a torsion pendulum, but not all authors would agree with the values they obtained (see Money et al. 1971). Quite recently in electrophysiological recordings from the squirrel labyrinth, Fernandez and Goldberg (1971) encountered deviations from the model which they attribute to adaptation of the sense organ and to a response to the velocity as well as to the displacement of the cupula.

Jones and Spells (1963) point out that the labyrinth of a fish is about twice the size of the labyrinth of a mammal of similar body size and, because the sensitivity is dimensionally dependent, they attempted to explain this significant size difference in terms of the type of head movements made by fishes when swimming. More recently, however, ten Kate, Van Barneveld, and Kuiper (1970) have shown that the large labyrinth size in fishes is a result of the way they grow and that canal sensitivities are very similar in all vertebrates.

The utriculus and the sacculus—Because the otoconia and the cupula membrane are about twice as dense as the endolymph, the otolith organ functions as a differential density accelerometer in responding to linear accelerations (Trincker 1962). Once again, elasmobranch preparations have

permitted a direct approach to the properties of the otolith organs, which in other vertebrates are experimentally unapproachable. In the otic capsule of the ray, Lowenstein and Roberts (1949, 1951) were able to perform a systematic analysis of the end organs. They found that all three organs (the maculae of the utriculus, sacculus, and lagena) responded to linear accelerations when exposed to fore-and-aft tilting. The utriculus had the most general response, for the macula sacculi contained gravity receptors only in its posterior portion, with the anterior part responding to vibrations. The otolith organs effectively measured a change in the static position of the labyrinth. Some units discharged maximally when the head was tipped in one direction (Figure 14); others would respond to the opposite movement, while still others discharged whatever the direction of movement. The units of the lagena, however, discharged maximally in the level position, and rapidly adapted if the head deviated from this (Figure 14).
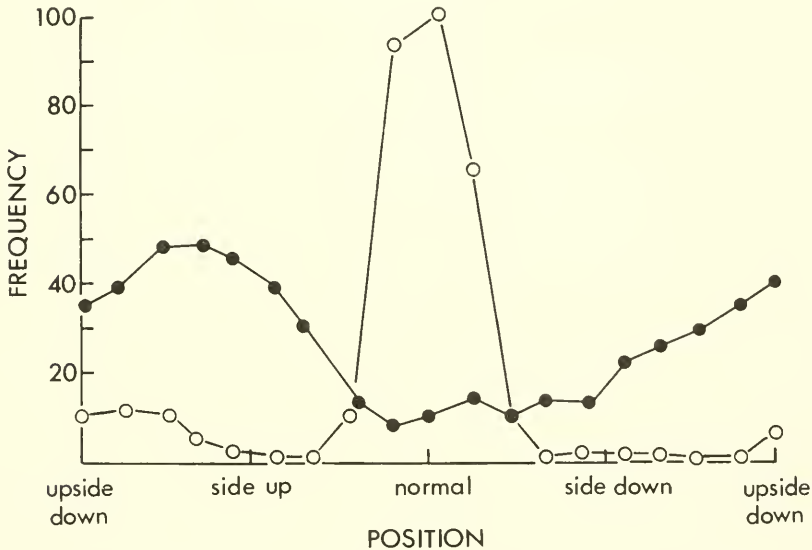
Figure 14   Frequency response (s^{-1}) of single units from the utriculus (●) and the lagena (o) of *Raja* in response to rotation of labyrinth (redrawn from Lowenstein and Roberts 1951*a*).

Lowenstein and Roberts (1951) found that units in part of the macula utriculi, the macula sacculi, and the macula neglecta would respond to vibration, following faithfully frequencies up to 120 Hz. The high vibrational sensitivity of the macula neglecta was particularly notable, and these authors suggested that it might be an important organ for sound detection, a role since advocated on anatomical grounds by Tester et al. (1972) and by Fay et al. (1974) who measured the microphonics for this receptor.

THE INNERVATION OF THE MECHANORECEPTORS

The tactile endings of the skin are supplied by nerve fibres carried in cranial nerve V and in the segmental spinal nerves. In contrast, the acoustico-lateralis receptors are innervated by cranial nerves (VII, VIII, IX, X). This important difference is illustrated in Figure 15, which emphasises that the lateral-line and auditory nerves project to a specialized brain centre—the acoustico-lateralis lobe—whereas the tactile input is widely distributed throughout the brain and spinal cord. It is not surprising to find, therefore, that the two systems generate quite different reflexes and behaviour patterns.
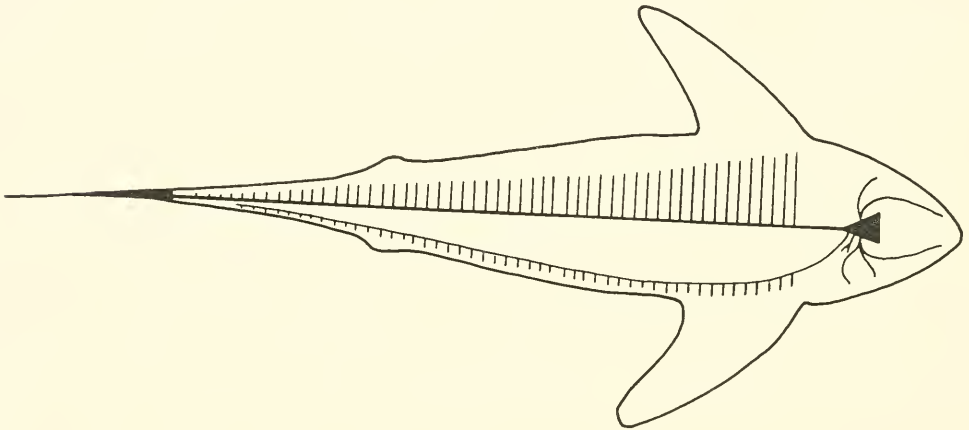
Figure 15   Diagram showing the pattern of innervation of the tactile sensory endings (top half) and the acoustico-lateralis system in an idealised shark.

Skin Receptors

The tactile endings of the skin of the head are supplied by cranial nerve V, which contains about 6000 sensory fibres in *Scyliorhinus*. Responses to mechanical stimulation recorded from branches of nerve V in the head of *Torpedo* (Platt et al. 1974) have shown that the skin receptors are highly sensitive to touch and respond well to "mild water flow."

The composition of the elasmobranch spinal nerves has been discussed by Roberts (1969b), who showed that in the sharklike fishes the motor and sensory fibres were held in separate bundles but that they were mixed together in a single bundle in the rays. Perhaps this is because in rays, as in most vertebrates, sense organs lie among the muscle fibres. Each dorsal root of a spinal nerve in *Scyliorhinus* contains around 500 fibres, not all of which will supply tactile or Wunderer endings. If every segmental nerve contained this number then there would be about 40,000 peripheral channels available to the skin receptors in the body; most of these fibres are 6 μm in diameter.

The area of the body supplied by one segmental spinal nerve—the sensory dermatome—has been determined for *Scyliorhinus* by Rijnberk (1904) and Roberts (1969*b*) (Figure 16) and for rays by ten Kate (1928). Unlike the motor nerves, which supply only adjacent motor segments (Bone 1964), the sensory fibres distribute over a number of adjacent segments, so that each dermatome overlaps with its neighbour.
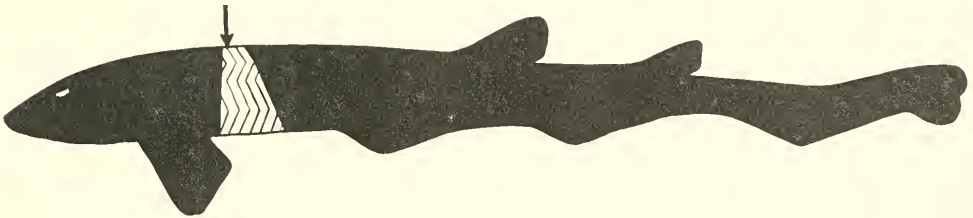
Figure 16   The form of the dermatome of one abdominal segment (arrow) of *Scyliorhinus*.   Note the trapesoidal shape and the extent of the dermatome. (Roberts 1969*b*).

Acoustico-Lateralis System

Sensory Centres of the Lateral-Line—Much of the earlier work on the anatomy of the cranial nerves of elasmobranchs was carried out in the cause of developing an evolutionary theory of the vertebrate head. Therefore, it does not deal specifically with lateral-line innervation, which is complicated by the parallel distribution of nerves V and VII and, in elasmobranchs, by the presence of the ampullae of Lorenzini.

An important step was taken by Marshall and Spencer (1881), who demonstrated that the buccal nerve, which supplies the infraorbital canal, was part of the facial nerve (VII) and was not a component of nerve V. This led to the conclusion that the trigeminal nerve (V) did not supply the lateralis system, and to Ewart's classification of the canals, based on their innervation, which we have already considered. Cole (1896) discusses some possible exceptions to this pattern of innervation.

An excellent description of the innervation of the head canals is provided by Norris and Hughes (1920) for *Squalus*, but the pattern is common to most elasmobranchs. The lateral-line organs of the head canals (and the ampullae of Lorenzini) are supplied by fibres of the anterior lateral-line nerves (superficial ophthalmic, buccal and external mandibular branches) which are intermingled in nerve VII with the fibres serving other sensory modalities, and the lateral canal is innervated by the posterior lateral-line nerve, which enters the medulla in association with nerve X. The diagram of Figure 17 shows the arrangement in *Squalus*, where some of the lateral canal sense organs are supplied by nerve IX as well. There are about 6000 lateral-line fibres feeding into the lateral-line lobes in *Scyliorhinus*, mostly about 12 μm diameter; the cell bodies of these fibres cluster in ganglia peripheral to the brain.
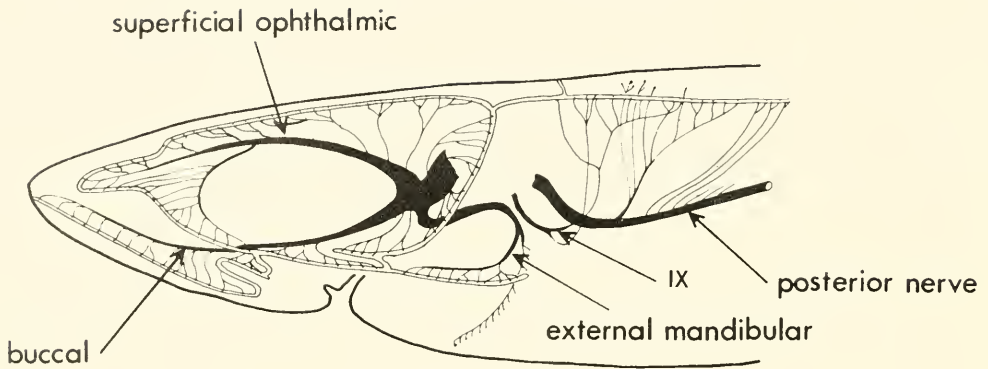
Figure 17   Innervation of the head canals of *Squalus* (modified from Norris and Hughes 1920).

The region of the brain that receives the lateral-line nerves is dominated by the cerebellum, which in these fishes consists of three portions: the unpaired corpus, the paired auricles, and paired lateral-line lobes (Figure 18).
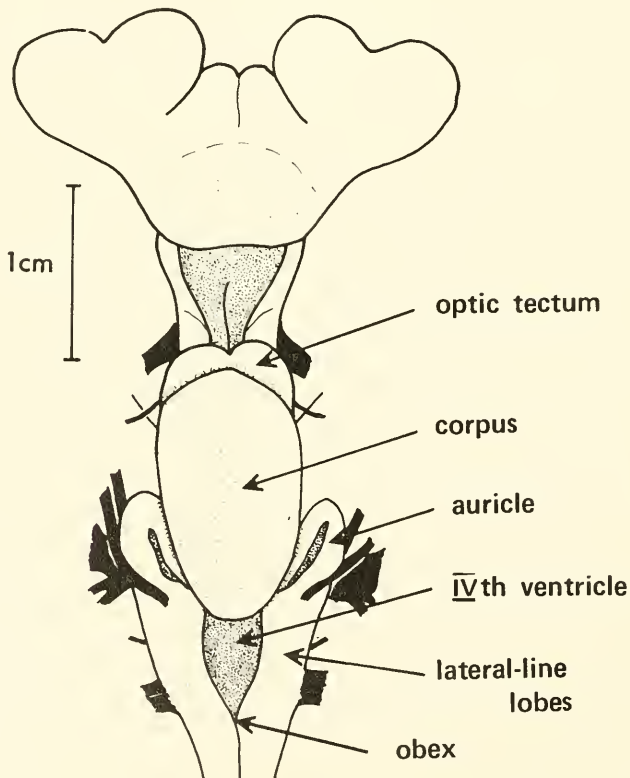
Figure 18   Dorsal view of the brain of *Scyliorhinus*.

The corpus is mostly concerned with spinal pathways, whereas the other two centres are specifically related to the acoustico-lateralis system.

Our knowledge of the projection sites is based on conventional neuro-anatomy (Kappers, Huber, and Crosby, 1936), on some degeneration studies (Campbell and Boord 1971), and on field and unit potential analyses (Ilinsky et al. 1971, Enin and Ilinsky 1972, Enin et al. 1973, Paul and Roberts 1976a).

The fibres of the anterior nerve enter the medulla and pass to a discrete medial lobe which overhangs the fourth ventricle, being separated from the medullary wall by a distinct cleft, and forms the *dorsal nucleus* or *anterior lateral-line lobe*. The posterior nerve enters with the fibres of the vagus at the rear of the medulla and ascends laterally almost as far as the auricle to give fibres to the *medial nucleus* or the *posterior lateral-line lobe* (Figure 19). Fibres from these lobes then pass to the auricles, while others, which do not enter the lobes, ascend to the *lateral cerebellar nucleus*.

The lateral-line projections in the dogfish have been recently described in detail by Paul and Roberts (1977a), who found that the secondary neurons of both lobes were large multipolar cells with spiny dorsal dendrites. These dendrites resemble the dendrites of cerebellar Purkinje cells in extending into the molecular layer, which consists of many unmyelinated parallel fibres and stellate cells. The obvious resemblance of this arrangement to the well-known organization of the cerebellum has lead to the widely accepted idea (Johnston 1902) that the cerebellum developed phylogenetically from the acoustico-lateralis centres. There are, however, important differences in the organization of the two structures. Nevertheless, as is revealed in Figure 19, the lateral-line lobes, the auricles, and the cerebellar corpus are superimposed on, and have neural circuits that are in parallel with, the hind brain centres.

The electrophysiological studies (Paul and Roberts 1977a) have revealed certain features about the kinds of analysis carried out by the lateral-line lobes. First, they show that the input is excitatory on the large secondary neurons so that even a single input is converted into a multiple discharge. The axons of these cells then project onto the extensive reticular system (Restieaux and Satchell 1956). Second, the failure of the lateral-line cells to follow faithfully any stimulus delivered more frequently than about 100 Hz, because of powerful inhibitory processes, shows that the lobe functions as a low-pass filter, even though the sensory fibres are capable of operating at a higher range. The final feature revealed by the electrophysiology is that the latency variation at the first synapse is large enough to prevent the lobe resolving small time differences between incoming signals.

The ventral dendrites extend widely and make synaptic connections with afferent fibres in a complex neuropil. The impact of the lateral-line input is therefore widely distributed throughout the lobe, and there is strong smoothing of the input. For example, the pronounced rhythmic activity generated in parts of the lateral line during ventilatory and swimming movement are out of phase and their impact on individual neurons will cancel out. However, natural external disturbances away from the fish cause an almost simultaneous signal to be set up along the transducer array, which will
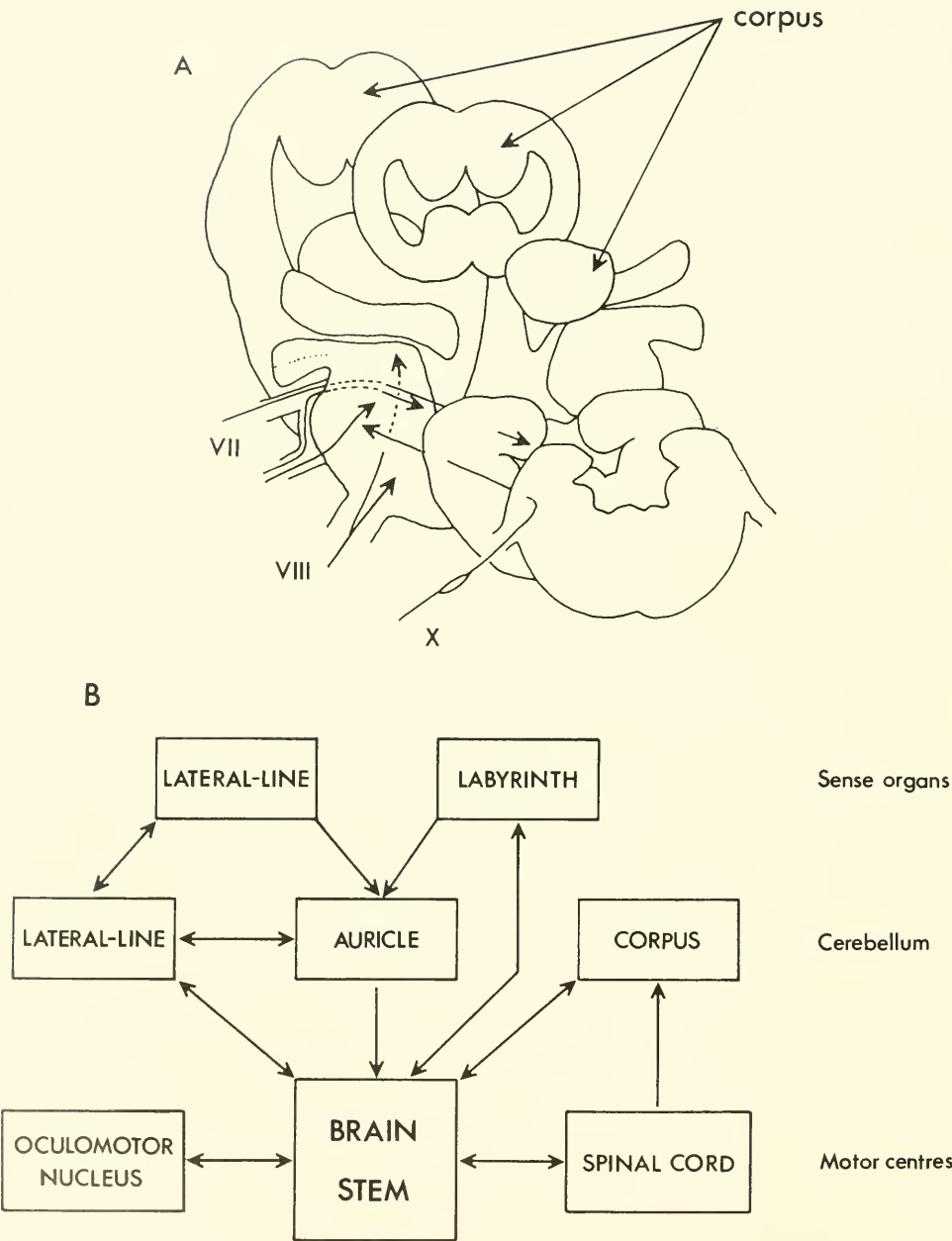
Figure 19 (A) Four representative transverse sections cut through the hind brain of *Scyliorhinus* to show schematically the projections of nerves VII, VIII, and X. (B) Schematic showing the relationship of the acoustico-lateralis sense organs, the components of the cerebellum, and the motor centres of the selachian brain.

facilitate and be passed by the secondary neurons. A steady input, even if not patterned, will nevertheless have an overall impact on the excitability of the secondary neurons, as was shown by Alnaes (1973*b*), who studied statistically the spontaneous discharge of the secondary cells in *Anguilla* and showed that this ceased immediately when the posterior nerve was cut.

Sensory Centres of the Vestibular System—The labyrinth receives its innervation from nerve VIII, which in elasmobranchs, as in all vertebrates, has two roots. The anterior root supplies the sense organs of the anterior vertical canal, the horizontal canal, and the utriculus. The posterior branch supplies all the other sense organs.

In the region of the entry point of the nerve, three vestibular nuclei can be recognised: the superior vestibular nucleus and the magnocellularis nucleus (Smeets and Nieuwenhuys 1976) and a ventral vestibular nucleus (Montgomery 1977). In a combined anatomical and electrophysiological study (Montgomery 1977), it was found that vestibular afferent fibres monosynaptically excite neurons in these nuclei.

Efferent Innervation of Acoustico-Lateralis Receptors—An important aspect of the innervation of many acoustico-lateralis receptors is the efferent supply. Efferent nerve fibres are absent from the labyrinth of *Myxine* (Lowenstein and Thornhill 1970), and from the lateral line of the lamprey (Yamada 1973), although not from the labyrinth (Lowenstein et al. 1968), and are rare in the eel (Yamada and Hama 1972). They have been reported so far for nearly all elasmobranch organs—the labyrinth (Lowenstein et al. 1964), the lateral-line (Roberts and Ryan 1971), and Savi's vesicles (Nickel and Fuchs 1974)—but have not been sighted in the pit organ or in the ampullae of Lorenzini.

Although efferent fibres have been detected at the periphery, both electrophysiologically and with the electron microscope, there is considerable uncertainty about the central location of their cell bodies, even in the well-studied case of the mammalian cochlea (see Klinke and Galley 1974). The possibilities for the elasmobranch lateral line have recently been examined by Paul and Roberts (1977*b*), who, using electrophysiological techniques, showed that the efferent neurons of the anterior lateral-line nerves are in the rostral region of the anterior lateral-line lobe. By backfilling the axons of these cells with cobalt salts they were able to identify them as multipolar neurons, with axons that entered the lateral-line nerve bundle.

The impact of efferent nerve activity—Wherever it has been studied it has been shown that stimulation of the efferent fibres leads to an inhibition of impulse activity in the primary afferent fibres. For example, in *Scyliorhinus*, electrical stimulation of the efferent fibres has a clear effect on the afferent discharge, the outcome depending on whether the unit is spontaneously active (Russell and Roberts 1972). If, as is generally the case, the afferent unit is discharging spontaneously, efferent stimulation reduces or inhibits this discharge during the stimulating burst and for up to 200 ms afterwards (Figure 20). The inhibition is usually evident only if the stimulating
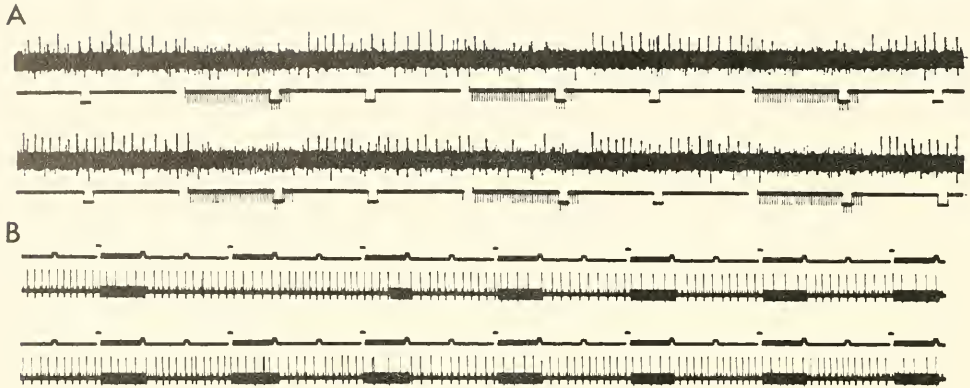
Figure 20 Impact of efferent nerve action on spontaneous afferent activity (*Scylio-rhinus*). (A) Total suppression of spontaneous afferent impulse activity brought about by electrical stimulation of efferent nerve fibres; the upper trace shows afferent unit activity, and the lower trace shows the stimulus given to the efferent fibres (in this case a train of pulses at 42 s^{-1}). (B) Samples of afferent unit activity, showing the impact of efferent nerve stimulation (marked on upper trace) at 100 s^{-1} (Russell and Roberts 1972).

bursts contain high-frequency shocks (>40 s^{-1}), but effects with lower frequencies have been seen. Successive stimulations have a declining impact (Figure 21), perhaps because of "fatigue" at the efferent terminals. If the units show no resting activity a stimulating train to the efferent nerve is followed by a brief afferent discharge (three or four impulses) about 500 ms after the stimulus train has ceased; this discharge is presumably a postinhibitory rebound.
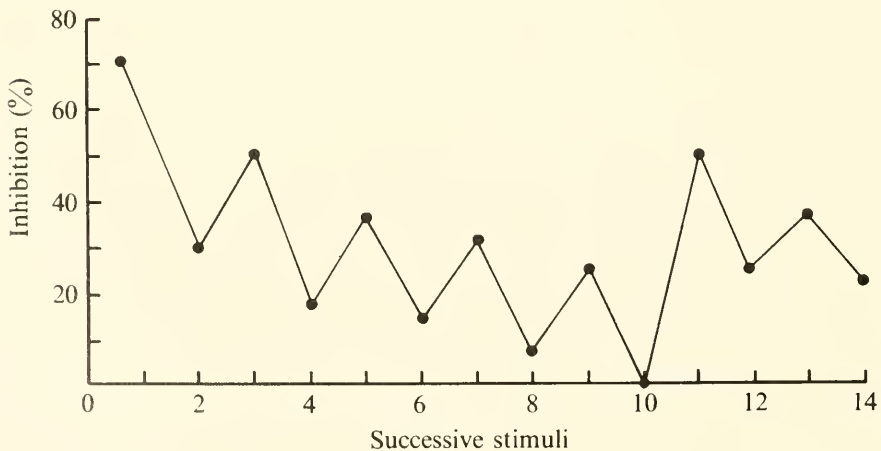
Figure 21 Variability and decline of the inhibitory effect of electrical stimulation of efferent fibres in *Scyliorhinus*. Complete inhibition (100%) indicates that the unit was totally inactive during the stimulation period; at 0% the afferent fibre was discharging at its resting frequency (15 s^{-1}) (Russell and Roberts 1972).

The time of efferent nerve activity—The observation that the efferent neurons reside in the lateral-line lobes might imply that the efferent system forms a feedback loop so that strong stimulation of the hair cells would achieve a modifying inhibition. However, at present there is no unambiguous evidence for this type of circuit. Certainly, electrical stimulation of the afferent fibres will provoke an efferent discharge (Roberts and Russell 1972; Paul and Roberts 1977*b*), but natural lateral-line stimulation does not evoke efferent nerve activity (Figure 22B). Indeed experiments designed to examine the relationship between natural lateral-line stimulation and efferent activity showed the ineffectiveness of lateral-line stimuli (Roberts and Russell 1972) and gave the clear impression that the type of stimulus that was successful in evoking efferent action (mostly vestibular and touch) was normally followed by some movement of the fish (Figure 22C). In the shark-like elasmobranchs these movements depend on two muscle systems that are brought into action at different times—steady, rhythmic movements involving only the peripheral red muscle system and briefer, larger unsustained movements, such as "escape" movements, produced by the extensive white musculature.

Vigorous brief movements of the fish are a frequent response to strong tactile stimulation and are immediately preceded by and accompanied by activity of the efferent neurons (Figure 23A, 23B). At these times there is a general correlation between the frequency of the efferent nerve activity and the amplitude of movement. The movement of the body alone, though, is insufficient to stimulate the efferent system, because passively induced body movements are not accompanied by efferent activity (Figure 24C). The efferent neurons are spontaneously active, discharging a few impulses at low frequency (5–10 imp/s), during rhythmical swimming movements that involve red muscle fibres (Figure 23C, D).

The consequence of efferent nerve activity—The demonstration that electrical stimulation of the efferent fibres at frequencies above 40 Hz causes a pronounced inhibition of lateral-line activity, as well as the finding that efferent discharge frequencies of this order are obtained naturally in actively swimming fish, implies that a swimming dogfish should show reduced sensitivity to lateral-line stimulation. This has been directly tested by Russell and Roberts (1974), who recorded total nerve activity from the intact buccal nerve, set up in response to a vibrating probe placed close to the head canals in stationary and in swimming fish. Figure 24 shows that the amplitude of the response in swimming fish is attenuated by as much as 50% and that this reduction is absent when the lateral-line nerve is transected centrally, to eliminate the efferent downflow.

A theory of efferent nerve action—These experiments on the timing of the efferent activity have clearly established two important points relevant to efferent function: (1) a true feedback system cannot exist, and (2) efferent activity is closely associated with body movement.

During steady movement it is now clear that the efferent system cannot function to counteract the rhythmical afferent activity generated during
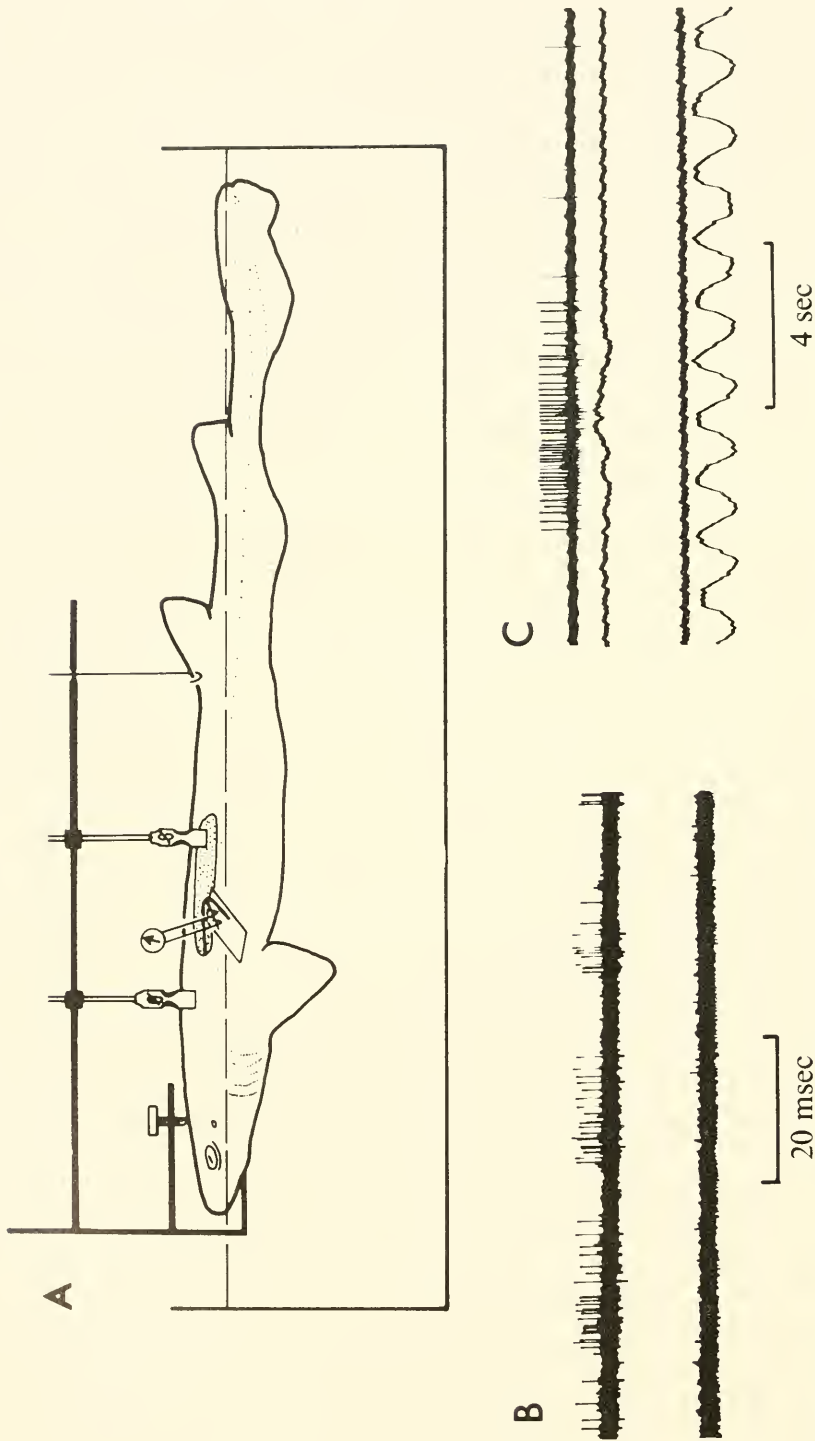
Figure 22 (A) Diagram of the experimental preparation for studying lateral-line efferent activity in the swimming dogfish (*Scyliorhinus*). A decerebrate fish is clamped firmly at the head, and fine branches of the posterior lateral-line nerve are placed over recording electrodes. (B) Efferent unit activity recorded in response to tactile stimulation (top trace) and lateral-line stimulation (bottom trace). (C) Top traces show the activity of an efferent fibre at the time of body movement, indicated by the transducer record. Bottom traces show that passive movement of the body is ineffective in generating efferent nerve activity (Roberts and Russell 1972).
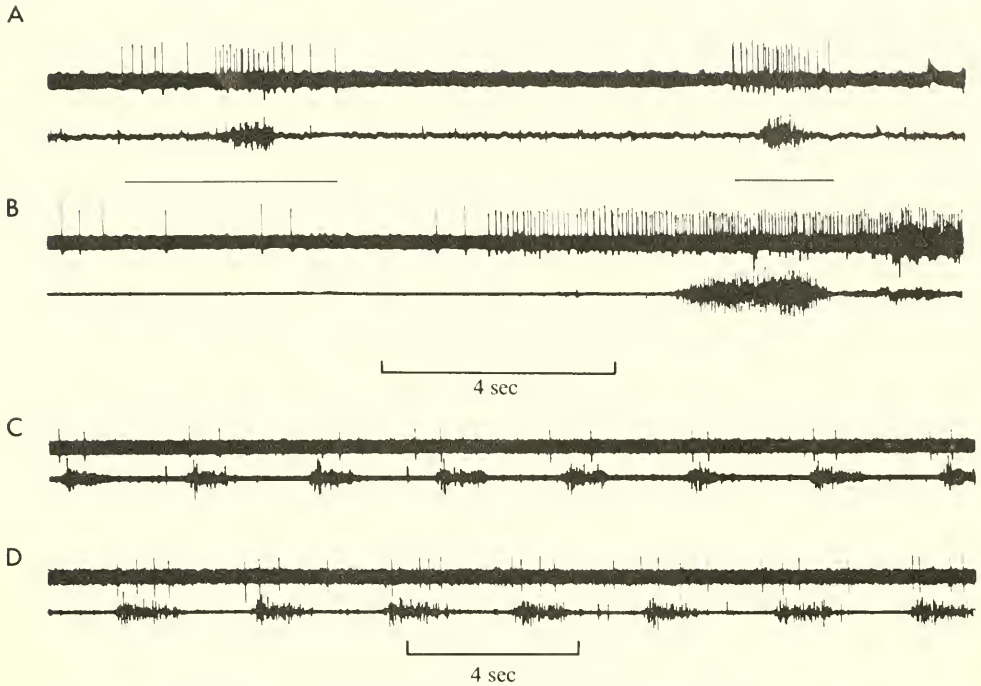
Figure 23   Efferent nerve activity accompanying body movement. In all records the bottom trace is the electromyogram recorded from muscle fibres of the body; The top trace is the record of an efferent fibre. (A) Responses to tactile stimulation to the body (marked by horizontal bars). (B) Vigorous tactile stimulation is accompanied by large movements and high-frequency efferent discharges. (C, D) Rhythmic activity of the efferent system accompanying steady swimming movements recorded from two fish (Roberts and Russell 1972).

swimming (see Roberts 1972 and later), for, not only do the efferent fibres discharge at such a slow rate, but also, very careful patterning of the discharge would be needed to ensure that the efferent impact coincided with the stimulus evoked by movement for all frequencies of locomotion. In fact, the evidence suggests that all the efferent axons discharge simultaneously.

During violent movements, in either escape or attack it is clear that the efferent discharges do achieve rates sufficient to reduce sense organ sensitivity. As it is also evident that the lateral line is strongly stimulated (Roberts 1972) during movement, it has been suggested (Russel 1971a; Roberts and Russell 1972) that the function of the efferent system might be to prevent short-term fatigue occurring in the receptors, so that the lateral-line system would be immediately responsive the moment any violent movement ceased (Figure 25). In the case of *Scyliorhinus*, an escape movement provoked by (say) a pinch on the tail consists of a few brisk movements followed by a glide, and we would expect the lateral-line sensitivity to be reduced just before and during the tail beat but to have returned to normal for the glide.
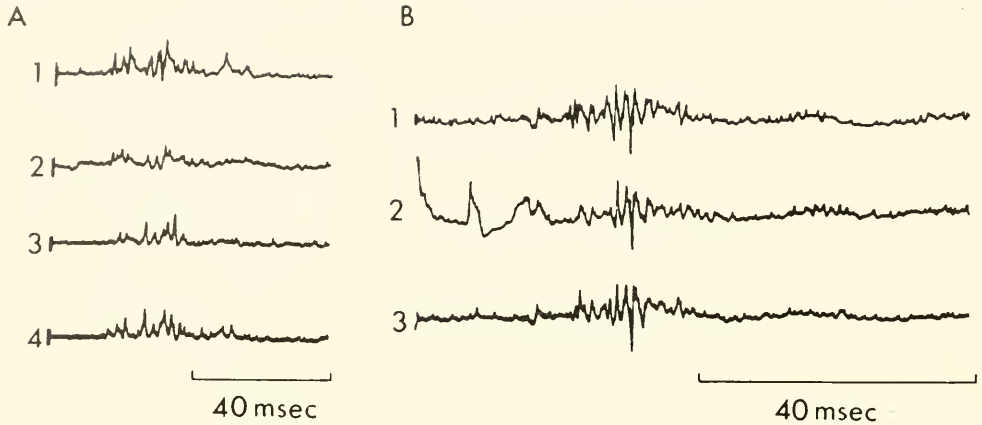
Figure 24   Total lateral-line nerve activity in response to head lateral-line stimulation of dogfish (*Scyliorhinus*). Traces A1, A4, B1, and B3 were recorded when the fish was stationary. A2 and B2 were recorded during violent movement and A3 during slow, steady swimming. All traces in B were taken after the nerve had been cut centrally to eliminate any efferent nerve impact (Russell and Roberts 1974).
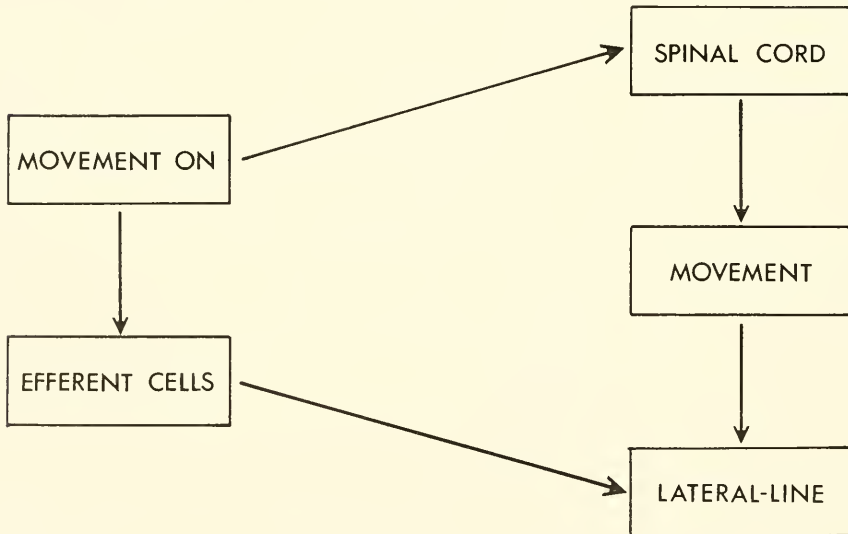
Figure 25   Schematic showing how the movement centres of the hindbrain and efferent neurons discharge concurrently and bring about an inhibition of the lateral-line hair cells during body movement.

A direct test of this theory remains to be performed, although it should not be difficult to see whether lateral-line organs are protected by the efferent fibres. Certainly, strong stimulation has a powerful action on lateral-line activity, for, as is shown in Figure 26 for a single unit lacking an efferent supply, strong mechanical stimulation is followed first by complete
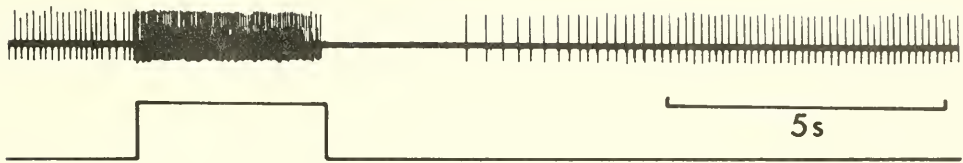
Figure 26 Single unit of posterior lateral-line nerve of *Scyliorhinus* exposed to strong mechanical stimulation for duration indicated by horizontal bar. When the stimulus ceased, spontaneous activity was absent for 2.5 s.

cessation of spontaneous activity and then by a low discharge rate, with great variability.

Efferent activity in the vestibular system—At present no data are available from the elasmobranch labyrinth on the role of efferent activity, although from the limited information now available on the vestibular efferent fibres of the frog and goldfish, discussed by Klinke and Galley (1974), there also appears to be a relationship between the efferent system and body movement.

A recent claim has been made in the case of the labyrinth of the frog that "receptor-to-receptor" fibres are present and promote sensory interaction between adjacent receptors (Gribenski and Caston 1974). Interconnecting fibres, initially on the basis of light microscopy (Peters 1971) but now on the basis of electrophysiology and the electron microscope (Späth and Lehmann 1975), have also been claimed for the lateral-line system. If these claims are substantiated the problem of afferent coding in these systems becomes much more complicated than at present thought; further examination of these difficult problems is urgently needed.

The Significance of Mechanoreceptors in the Behaviour of Elasmobranchs

It is very difficult to assess correctly the biological function of a particular sense organ. For olfaction, vision, hearing, and touch we have our own subjective experiences, which can at times be applied to comparative animal studies but provide no clues about sense organs we lack. In such circumstances, as with the lateral-line system, do we invoke an additional sensation, a sixth sense, as was suggested by Leydig?

The basic experimental approach to the problem of assigning function to a sense organ system is to observe the impact on behaviour of (1) removal of the sense organ or (2) its denervation ("de-afferentation"), usually accompanied by (3) controlled natural stimulation or (4) electrical stimulation of sensory nerves. No single method is individually conclusive, but taken together with the results from electrophysiological studies, which delineate the likely modality of the system, these methods give an insight into an animal's sensory capabilities.

The first approach, the ablation or denervation of a sense organ or its brain centre, has been much favoured, but of all the methods it is perhaps

fraught with the greatest difficulties and complications. It has not always been appreciated that the *absence* of a sensory signal may be a major *positive* trigger to the nervous system, so that dramatic changes may result simply from elimination of an organ. This point is well illustrated by the acoustico-lateralis system; although removal of the lateral-line has no obvious effect, removal of a single labyrinth has an immediate impact on a shark's equilibrium.

The notable feature of the tactile reflexes in elasmobranchs is that the response is immediate and local, although in the case of strong stimulation the effect may spread and result in contraction of the white musculature and an overall change in the fish's behaviour. In contrast, the reflexes of the acoustico-lateralis system always affect the whole body and lead to major changes in the fish's orientation.

Tactile Reflexes in Elasmobranchs

For the most part the tactile endings are sparsely distributed, but areas of enhanced sensitivity exist on all the fins, particularly the pelvic and caudal fins, and around the head and jaws. It has often been reported that a feeding shark will "bump" possible prey with its snout, presumably to assess texture, and this is done with the sensory endings of nerve V. Nerve V endings are also involved in another aspect of feeding behaviour in which contact with the teeth evokes a fast contraction of the jaw musculature. This is brought about by the stimulation of receptors sited under and around the teeth which discharge along fibres that project to the mesencephalic Vth nucleus and have collaterals connecting monosynaptically with the Vth motor nucleus that provokes jaw contraction (Roberts and Witkovsky 1975).

The reflex responses of the fins to touch have been described by Lissmann (1946a) and Roberts (1967). Usually touch evokes only a local contraction, but strong stimulation of the base of the fins may affect swimming movements, which are inhibited and may not recover spontaneously. Stimulation to the tip of the caudal fin is a very effective stimulus for evoking large-amplitude rapid movements (Gray and Sand 1936) which result from the switch from the red to the white musculature (Bone 1966; Grillner 1974). The level of mechanoreceptive input is very significant, therefore, in switching from one muscle system to the other, although presumably this can also be achieved by descending central pathways. In the electric ray, *Torpedo*, although electric discharge is given to tactile stimuli applied all over the body, touch to the tail is always followed by a large discharge and by a vigorous turning movement that rotates the ray to face the provoker (Roberts 1969e).

Reflex responses to touch on the body have been studied in the "spinal preparation" by Le Mare (1936), Gray and Sand (1936), Lissmann (1946a), and Roberts (1967). These studies have shown that a vigorously swimming preparation will respond to a gentle ipsilateral stimulus with a sustained ipsilateral contraction, moving the body away from the stimulus. The amplitude of the swimming beat also increases, although if the stimulus persists it

may actually decrease. Ten Kate (1934) showed that these reflexes were initiated and controlled from within one body segment.

The Labyrinth in Equilibrium

Because of their accessible labyrinths, because of their hardiness, and because of the simplicity of their behavioural responses, the elasmobranchs were favoured experimental animals in early studies on labyrinth function. The range of experiments performed in several elasmobranch species and the significance of the results of the work of Loeb, Lee, Lyon, Kriedl, and himself are reviewed by Maxwell (1923) in his book *Labyrinth and Equilibrium*.

When the body of a shark is rotated (as in "roll") the eyes rotate to preserve the original visual field and the pectoral fins move to restore the body's position. Thus, if the body moves down on the right, the right eye will move up and the left eye will roll down, but the right pectoral fin will move down and the left will come up. These compensating movements, which show the interrelationships between the eye, fin movements and labyrinth position, require labyrinthine measurements of out-of-true positions.

The fin movements are to be interpreted in relation to the role of the fins in locomotion, for most elasmobranchs are denser than seawater and need to set their pectoral fins with sufficient angle of attack to generate the correct dynamic lift (Harris 1936). Measurement of the body's relevant angles is presumably done by the labyrinth.

Harris (1965) has described the movements of the eyes of swimming dogfish and related these to labyrinth function. He found that the eye movements of swimming dogfish (*Squalus*) did not fully compensate for the lateral movements of the head that occur during swimming; the head moved through an arc of about 25°, but the eyes moved backwards only 15°. He analysed these movements into a number of components and found that a free-swimming dogfish with intact labyrinths, but with the spinal cord cut (i.e. a spinal preparation), moved its eyes to obtain complete compensation, whereas in a fish with both eighth nerves cut, but with the spinal cord in connection with the brain, the eyes moved in the opposite sense to compensation; the combination of these opposing effects resulted in only partial compensation. Harris pointed out that this would stabilize a visual field on a plane close to the fish, whereas with total compensation the visual field would be stabilised at infinity.

Destruction of one labyrinth has a pronounced effect, causing movements of fins and eyes, but removal of both labyrinths causes no obvious effect. Surprisingly, fish thus altered swim in what appears to be the normal fashion.

The Lateral Line in Equilibrium

One role that it was soon agreed was not the function of the lateral line was an immediate coordination of movement, for lateral-line nerve section had

no impact on movement. Also, of course, the spinal dogfish, in which the brain had been destroyed and therefore the lateral-line centres obliterated, swam steadily. Nevertheless, all neurophysiologists who have worked with the isolated lateral-line organ have been impressed by its extreme sensitivity and by the obvious fact that body movements must have an excitatory impact. This problem was examined in the dogfish by Roberts (1972), who showed that, as expected, the lateral line was indeed stimulated by locomotory movements, for whereas in a stationary fish the lateral-line organ would be discharging steadily, in the swimming fish (the continuous condition in many open-ocean sharks) the swimming stimulus came to dominate totally lateral-line activity, which consisted of bursts of activity separated by periods of silence (Figure 27). At the time of this study it was not known how much the efferent activity would modify this locomotory discharge, but we now know this impact to be insignificant. Stimulus detection in these fishes therefore must be carried out against a continuously modulating background.

Recordings taken from the swimming dogfish show that certain features of the rhythmical discharge are correlated with body movements, so that the lateral line is capable of providing proprioceptive information. It does not follow, of course, that this information is used by the fish, for it is clear from what is known about the properties of the lateral-line lobe that much of this pattern would be lost even at the first synapse, because of the widespread smoothing and because of the low-pass properties of the centre. It is most likely, therefore, that the impact of the lateral line is that of a sustained tonic input.

The Role of Proprioceptors in Coordination of Movement

The importance of mechanoreceptors for the coordination of body movement in animals is a perennial topic for debate among comparative physiologists and has at times featured work done on the elasmobranch fishes. The
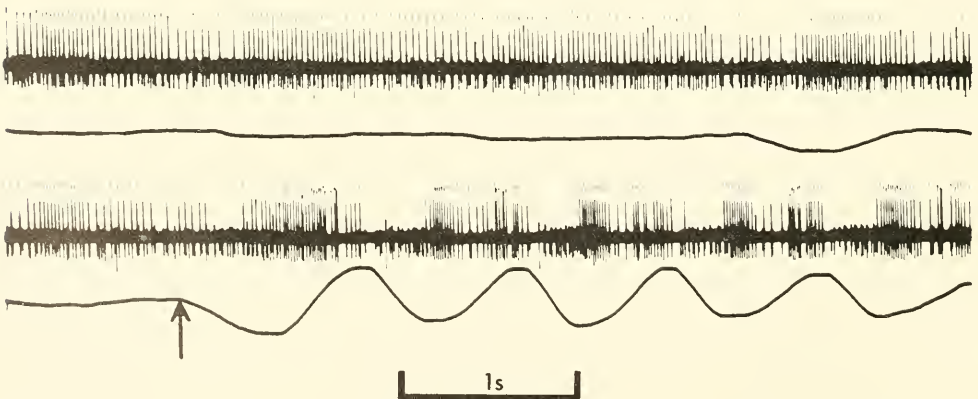
Figure 27 Continuous record of activity of single lateral-line unit in a dogfish that started to swim (at arrow); bottom trace is from the movement transducer (Roberts 1972).

value of the sharklike fishes in this area of research has lain in their rare capacity for performing regular swimming movements after complete removal of the brain. Consequently, and in complete contrast to other vertebrates, the elasmobranch spinal preparation is one whose locomotory movements are coordinated solely by spinal cord neurons.

Since persistence of movement in spinal dogfish was first observed at the end of the last century several workers have examined this preparation. The work of Gray and Sand (1936), among others, was important in showing that spinal neurons are capable of generating rhythmical activity even in the absence of proprioceptive input, although Lissmann's de-afferentation experiment (Lissmann 1946b) implied some role for proprioceptive feedback.

These experiments have contributed to arguments as to whether locomotion in vertebrates is governed by "central rhythms," generated by "oscillators" or produced by "chain reflexes," established by proprioceptive feedback. This debate, which is reminiscent of the polarised views seen in other disciplines (such as the preformation-epigenesis controversy of embryology and the nature-nuture debate of geneticists), in retrospect can be seen to have been rather sterile and notable for lack of definition. Indeed, in view of current ideas of corollary discharges and efferent supply of sense organs, the distinction between "central" and "peripheral" becomes very blurred, and the two extreme viewpoints are seen to be untenable. No movement could be completely determined by the sensory input, which would make the nervous system redundant, any more than the central nervous system could be totally independent. A more illuminating approach to the role of sensory activity in movement control is to attempt to define the activity of specific sense organs during movement and to determine the functional value of this activity.

All the evidence so far for the sharklike elasmobranchs indicates that the amplitudes and frequencies of the swimming movements are probably regulated by two separate but interrelated mechanisms. Also, whereas the sensory input, in one form or another, is important in sustaining the amplitude, the frequency is determined more by the properties of central neurons, which, depending on their excitability, tend to discharge spontaneously. Recordings taken from the spinal nerves of curarized spinal fish have shown that pronounced rhythmical discharges are obtained from the motor nerves (Roberts 1969a, Grillner, Perret, and Zangger 1976). These discharges are sustained even in the absence of proprioceptive activity and can be restarted after they have waned by the application of nonphasic tactile stimulation (Figure 28). These data indicate, not surprisingly, that much of the pattern of movement is determined to a large extent by the spinal cord. Nevertheless, changes in the timing of the proprioceptive feedback indicate that this too must have some role, for when the body of a free-swimming spinal dogfish is subjected to forced oscillation, the electromyographic records from the swimming musculature show very clearly that the motor pattern becomes immediately entrained to the applied rhythm (Figure 29).

These two pieces of evidence can be incorporated in a general view of proprioceptive function. This requires only the assumption that the tendency
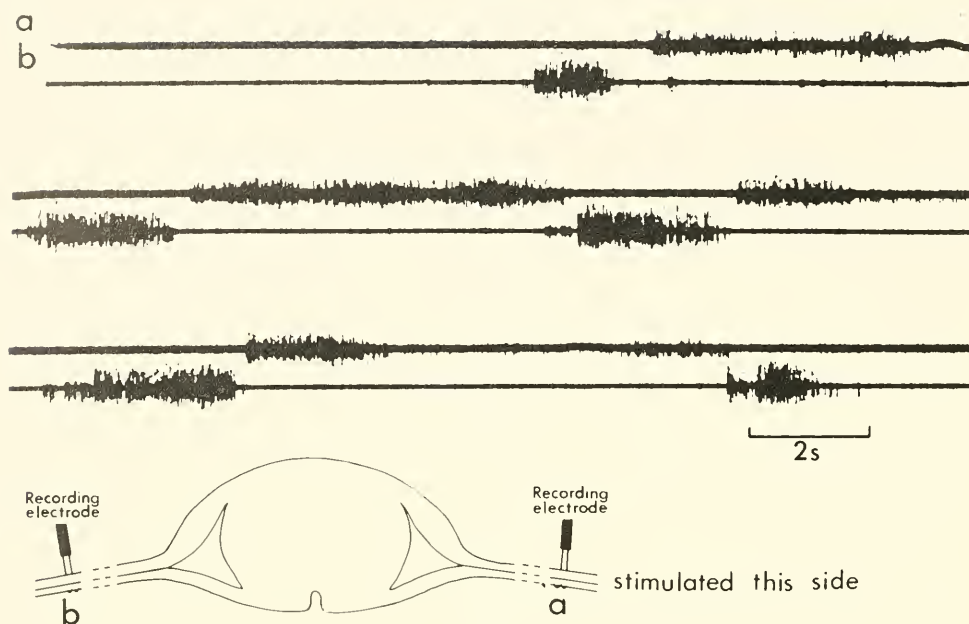
Figure 28   Whole nerve recordings from left and right spinal nerves of an abdominal segment in a curarized spinal preparation of the dogfish *Scyliorhinus*. The spontaneous rhythmic discharges had ceased but reappeared in response to strong tactile stimulation on the right side. Time scale—2 s (Roberts 1969*a*).

of the spinal neurons to discharge rhythmically is stabilised by proprioceptive feedback during movement so that these neurons, which are notable for their extensive dendritic arborizations and, presumably therefore for an extensive synaptic input, are kept from oscillating widly (Roberts 1969*d*).

Role of Lateral Line and Labyrinth in 'Hearing'

Any attempt to relate elements of natural behaviour to specific mechanical stimuli is made difficult in fishes by the presence of the lateral-line system. Were this absent, it could be assumed that the detection of vibrations set up some distance from the fish would be the role of the ear, while the skin tactile endings would respond to water currents and touch; but with the addition of the lateral line, such a simple division is not possible.

Electrophysiological experiments show that the lateral line is very sensitive to water displacements. The recognition of these disturbances when created by nearby moving objects should be useful for the discovery of prey, enemies, and sexual partners. Perhaps it aids in schooling, but whether in fact the lateral line has this role, and just what biological stimuli are detected by the system, have been the subject of much debate and testing. Since Parker's (1905) report that behavioural responses could be triggered by a tuning fork, some workers have maintained that the lateral line is sound-sensitive, whereas others have strongly opposed this view; these opinions are
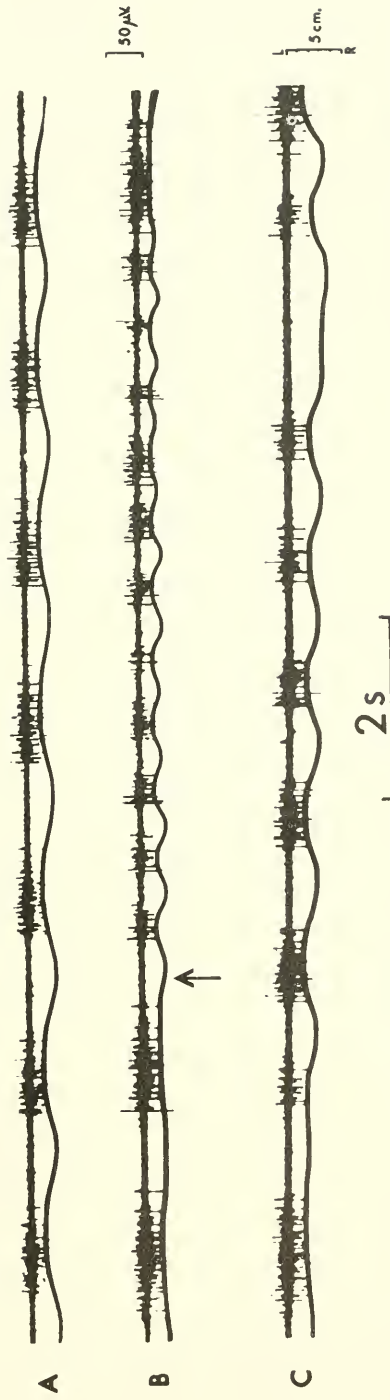
Figure 29 Impact of changes in timing of proprioceptive activity on muscle activity in swimming dogfish. (A) Electromyogram from the red musculature, showing the pattern of activity in steadily swimming spinal dogfish. (B) At point indicated by arrow the body was oscillated at a frequency greater than the swimming frequency. Note in C that when the oscillation slowed, the discharge was also delayed.

well covered by Dijkgraaf (1963a) and by some of the authors in Cahn (1967).

There is now considerable anecdotal evidence that sharks are strongly responsive to disturbances created in the water, but the type of response seems to depend on the shark, the disturbance, and the observer! For example, some divers (e.g., Eibl-Eibsfeldt and Hass 1959) believe that a loud shout made underwater will deter a shark attack (the "scream theory"); others regard this advice as "little short of criminal" (Cousteau and Cousteau 1970) because they believe that sharks will actually be attracted. The fact that sharks are lured by underwater disturbances has been noted for some time and has apparently been exploited by Solomon Island fishermen who attract sharks by creating noises with coconut shells that are said to simulate the clapping sounds made by lobster tails (Coppleston 1962)! The widespread observation by divers that sharks respond rapidly to struggling speared fish has resulted in recent attempts to examine the acoustic responses of sharks. This type of research is hampered, however, by the difficult technical problems associated with underwater sound, the large size of the fish involved, and the difficulty of establishing just which is the responsive sense organ. Much of this work, which is reviewed in detail elsewhere in this volume can, for present purposes, be summarised as follows:

1) Studies on sharks housed in small tanks: (*Mustelus* (Parker 1909); *Scyliorhinus* (Dijkgraaf 1963b); *Negaprion* (Wisby, Richard, Nelson, and Gruber 1964; Nelson 1967b))

2) Studies on large sharks in pens (*Carcharhinus* (Kritzler and Wood 1961); *Carcharhinus* and *Sphyrna* (Davies, Lochner, and Smith 1963))

3) Field studies, by divers or with underwater television systems, made on numerous species (Banner 1968; Richard 1968; Myrberg, Banner, and Richard 1969; Myrberg, Ha, Walewski, and Banbury 1972; Nelson 1967a; Nelson, Johnson, and Waldrop 1969; Nelson and Johnson 1972).

When taken together, these studies have revealed that sharks respond to low-frequency vibrations (<1000 Hz), which if pulsed can be strongly attractive (see Nelson and Johnson 1972). Such detection can certainly take place over long distances, for Nelson (1969) reports a response by a shark 183 m from a sound source, but as very few of these studies, except for those in group 1, have been combined with nerve or sense organ ablation, it is not possible to designate the responding system. However it should be possible, on the basis of what is known of the lateral line and the ear, to speculate reasonably about the likely roles of each sense organ under field conditions.

A clearer understanding of the types of disturbance created by an object moving in water has come from the writings of Pumphrey (1950), Harris and van Bergeijk (1962), Harris (1964), and van Bergeijk (1964). An object moving in seawater sets up simultaneously displacement and compression waves. These two disturbances are called *near-field sound* and *far-field sound* because the amplitude of the near field declines more, the greater the distance from the source. The near field is the dominant stimulus at distances

closer than 0.2 wavelengths from the vibrating source, whereas at greater distances only the far field would be important. By applying this idea, and recording the microphonic potential from the canals of *Fundulus*, Harris and van Bergeijk (1962) neatly demonstrated that the lateral line responds only to water displacement.

This does not mean, as has often been thought, that the lateral line detects only objects close to the fish for of course, as van Bergeijk (1964) emphasised, displacements occur at all distances between the source and the receptor. When the extreme sensitivity of the hair cell is taken into account, it is probable that the range of these sense organs is in fact much greater than is usually appreciated. Furthermore, the overall sensitivity of the system is certainly greater than that of individual organs because the signal-to-noise ratio can be improved greatly by central averaging. In the electric fishes, for example, the behavioural measure of threshold is about 200 times better than the thresholds determined electrophysiologically for individual organs (Machin 1962).

Because the lateral line and the ear are of very distinct design, their operational capabilities and individual responsiveness to pressure and displacement would be expected to differ. It is very unfortunate that, although the mechanics of the semicircular canals have been well studied, the hydrodynamic properties of the lateral-line system have not yet been examined. The lateral line of sharks, though it involves a canal system, is open to the sea, and its endolymph probably has physical properties similar if not identical to those of seawater. Most biologists believe that a displacement in the seawater directly disturbs the canal fluids, although how this takes place in fishes with closed canals is unclear. However, except for the endolymphatic canal of elasmobranchs, the ear is closed to the seawater and the internal fluids cannot be agitated directly by an external displacement. Instead, it is assumed that the whole fish vibrates slightly to a sound source and the hair cells are stimulated because of the difference in density between the otolith organs and the fish.

The displacement of the labyrinthine hair cells will certainly be out of phase with the external signal, and the response of the hair cells will be governed by the "stiffness" and "damping" of the system, characteristics which in turn are very dependent on the mass of the otolith. It is perhaps significant therefore that the otoconia of sharks vary in size even for one neuromast organ; perhaps in this way some "tuning" of the sense organs would be established which is essential in these fishes, where it is improbable that any nerve fibre can carry impulses faster than 300–400 Hz, but in which behavioural responses to frequencies higher than this have been observed.

An obvious pressure transducer is to be found in those bony fishes in which the swim bladder is coupled to the ear, but no comparable structures are found in elasmobranchs and it is uncertain whether they can respond to the pressure component of a sound. A recent finding of Fay and Popper (1974), if applicable to sharks, would suggest that the ear alone responds only to displacement. They were able to show by recording labyrinthine microphonic potentials in goldfish that in fish with the swim bladder

removed the response was to particle displacement, whereas when the swim bladder was present not only were the fish more sensitive but the response was coupled to pressure.

We must assume that high-frequency analysis is the function of the ear because all the evidence to date indicates that the lateral line works only at low frequencies. Thus, apart from the electrophysiological data already discussed, which demonstrated the low-pass properties of the brain centres, Parker's well-known experiment (Parker 1905), in which he obtained responses in *Mustelus* to a 6-Hz tuning fork, which were lost when the lateral-line nerves were cut, indicates a similar low-frequency preference. In *Acerina* (Kuiper 1967) and in goldfish (Weiss 1969), behavioural responses evoked by selective lateral-line stimulation were obtained only at low frequencies of stimulation (<200 Hz).

Myrberg et al. (1972) found that sharks sited in the far field of a vibrating source were well able to localize it. This observation would be difficult to explain if the sharks were responding to the pressure waves, because, as van Bergeijk (1964) has emphasised, in most fishes directional localization is probably only possible if use is made of an array of receptors such as the lateral line. In Myrberg's experiments the frequencies used were well within the range of the lateral-line organs and we must assume that the lateral line was detecting the displacement from the distant source; a most interesting experiment would be to test localization to far-field sounds of frequencies that lie outside the lateral-line range.

If, as we are suggesting, the ear also responds to displacement, then, in the case of a complex sound with components of different frequency, it should be possible to distinguish between a large distant object and a small object sited nearby, by comparing the responses of the ear and the lateral line and by measuring the frequency of the signal as well as the amplitude of the displacements.

The receptivity of the ear and that of the lateral line also overlap to some extent with tactile sensation. The significance of cutaneous sensation has been too often overlooked, despite Dijkgraaf's longstanding observation (Dijkgraaf 1950) that behavioural responses in *Gobius* to a 100-Hz tuning fork were obtained even after bilateral labyrinthectomy and with both lateral-line nerves cut. Similarly, Parker (1909) found that responses of *Mustelus* to a bang on the side of the tank, although lessened after both auditory nerves had been cut, were not obliterated until the lateral lines had been denervated and the skin procainized as well.

The response of sharks to currents (e.g. Hodgson and Mathewson 1971) is a good example of overlap between the senses, although the response is probably mediated only by the tactile endings of the skin. Parker (1905) found that both *Raja* and *Mustelus*, when exposed to a strong current, swam upstream even if the lateral line was denervated. Current orientation (rheotropism) is an example of a behavioural response in which the lateral line is *detecting* the stimulus but is not *monitoring* it; its response therefore does not initiate behavioural activity. Perhaps this is not surprising, for we have already seen that considerable smoothing takes place in the lateral-line lobes;

only simultaneous signals, differing in amplitude along the array, have an impact on the brain.

The association of the tactile receptors and the lateral-line endings is expressed in the distinction between "distant touch" and "touch" and in the fact that signals detected by the lateral line appear to be interpreted by a fish as arising from some external source, whereas touch is a sensation referred to the skin surface. We do not know how this perceptual difference is achieved, but it must result from the mode of neural organization. Pumphrey (1950) emphasised that the tactile responses were generated at the segmental level, whereas analysis of acoustico-lateralis function was carried out in a closely grouped medullary centre (see Figure 15). The enormous number of synapses made in the lobes, the integrative, wide sampling capacity of the secondary neurons, and the possibility of the auricular and the lateral-line centres interacting by means of the parallel fibre pathways, are all specialisations that are probably important in this type of analysis (Paul and Roberts 1976b).

CONCLUSIONS AND PROSPECTS

Our concluding view that the major mechanoreceptor groups provide a spectrum of sensation is not startlingly novel. More than 150 years ago Knox (1825) deduced that the lateral line contains "organs of touch, so modified however, as to hold an intermediate place between the sensations of touch and hearing." Since then, little progress seems to have been made in the analysis of fish sense organs, particularly in relation to behaviour. Many areas of research await exploration, therefore, but we can be confident that the elasmobranch fishes, because of their limited repertoire of behaviour when compared with the teleosts, and because of their suitability for experimental work, will provide useful research material. Three kinds of research problems come immediately to mind and are now beginning to be examined.

At the behavioural level an important problem concerns the significance of "hearing" in shark behaviour, which has begun to be described in the last decade and which should lead to vigorous research into the mechanisms of hearing and into the identification of attractive and repellant sounds.

At the neurological level, the reflexes associated with labyrinthine stimulation, because of their simplicity and because of the ease of access to the labyrinth—features that have made the elasmobranch favoured material in the past—should now attract neurophysiological investigation.

Finally, an important question in sensory physiology concerns the significance of the efferent system. This is a complex problem and will require comparative data from a number of species; once again, the elasmobranch labyrinth should prove particularly informative.

ACKNOWLEDGMENTS

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UNDERWATER SOUND—ITS EFFECT ON THE

BEHAVIOR OF SHARKS

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INTRODUCTION

Divers who frequent areas where sharks prevail have long known that spear-fishing greatly increases the chance of a shark encounter. Stories of such encounters often are surprisingly similar, regardless of geographical location. Usually sharks are not seen when the fish is speared but appear suddenly a few moments later. In the few cases in which their approach is noted, the rapidly moving predators often come from directions where chemical information could not possibly have reached them, due to the prevailing current or the extremely brief time between the spearing and their arrival. The approaching sharks appear well oriented; they locate the struggling fish within seconds, whether in the open or hidden in a recess of the reef. Such orientation appears clearly to depend, however, on the struggling movements of the speared fish. If the fish rests quietly, sharks only a few meters upstream show no interest.

These accounts, as well as those from the literature (e.g., Eibl-Eibesfeldt and Hass 1959, Hass 1959, Hobson 1963, Limbaugh 1963, Wright 1948), clearly suggest that sharks can, indeed, be attracted solely by the sounds of struggling fishes. This suggestion was first tested by Nelson and Gruber (1963, see also Wisby et al. 1964), who showed that the sounds of struggling produced by a speared grouper consist of rapid pulses of broadband noise with peak energy below 100 Hz and that similar sounds can attract several species of free-ranging sharks. Playback of rapid pulses of noise with frequencies below 60 Hz were especially effective in that regard, while sounds at these same frequencies but without pulsing or with pulsed noise bands between 400 and 600 had no effect.

These findings were discussed during the Second Symposium on Marine Bioacoustics at New York City in April 1966, when several speakers commented that attempts to confirm such attraction by a variety of underwater sounds had failed. These remarks immediately followed another discussion that ended with most of those present accepting the idea that fishes are incapable of orienting to a sound source located beyond the region of the near-field effect.[1] Since the published report on shark attraction included the important point that the rapidly approaching animals appeared well oriented to the sound source, such responses, if real, could have occurred only within the near-field of the sound. Yet the distances mentioned in the original reports (e.g., Wisby and Nelson 1964) strongly suggested that oriented movements had begun beyond that region. This difficulty, as well as the lack of confirmation, resulted in the opinion that confirmation of such results was

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[1]This effect consists of the relatively large amplitude excursion of the medium close to a sound source that is associated either with movement of the source in excess of the compressibility of the medium or with the curvature of the wave front. Attenuation of this effect occurs faster than does attenuation of particle motion associated with pressure fluctuations; thus the near-field effect predominates only within a region generally extending less than one wavelength from the source; beyond that point, one enters the far-field (see Banner 1972, van Bergeijk 1964).

needed before serious consideration could be given to the perplexing prob-
lem of far-field orientation by sharks.

Clear confirmation of those results appeared shortly thereafter when,
fortunately, field experiments were again conducted on several species of
sharks frequenting the underwater television site of the Bimini (Bahamas)
Video-Acoustic Installation (Myrberg et al. 1969, Richard 1968) and other
locations in Bahamian and Florida waters (Banner 1968, Nelson et al. 1969).
These independent studies established that certain kinds of underwater
sound are highly attractive to various species of sharks and that such attrac-
tion can be initiated in the far-field.

Generally, long periods of time must pass before results from field tests
can be reexamined by appropriate tests of confirmation. Fortunately this has
not been the case with this question even though tests have often involved
large, highly mobile animals. Various findings regarding the acoustic biology
of free-ranging sharks have been repeatedly confirmed by the independent
studies of Nelson and his coworkers, who used Pacific species (Nelson and
Johnson 1970, 1972; Nelson et al. 1969), and by those of our team, using
primarily Atlantic species (Banner 1968, 1972; Myrberg 1969, 1972; Myrberg
et al. 1969, 1972, 1975a, 1976). Such confirmation has assuredly posed
additional questions and hypotheses from knowledge only recently gained,
but it has been necessary, since the research has had implications regarding
human safety.

SHARKS AND SOUNDS—A STORY WITH MANY IMPLICATIONS

The story that has unfolded during the last few years regarding the effects of
sound on the behavior of sharks encompasses a wide variety of diverse yet
interrelated topics. They include biophysical and ecological considerations,
learning and orientation processes, and even neural events at the level of the
sensory receptors. To highlight the most interesting points, this part of the
report is divided into a number of sections, each centering on one topic. The
entire story, as we at present understand it, includes information extending
beyond what can be covered under a few arbitrarily chosen headings. There-
fore, where necessary, an attempt has been made to explain interrelation-
ships that bridge the topics covered. The first section deals with the types of
sharks that have been attracted to sound sources; the second and third
center on the physical factors that appear to be important or unimportant
for an acoustic attractant; the fourth highlights the behavior of sharks in the
vicinity of a sound source; the fifth deals with those qualities of sound that
apparently promote a response opposite to attraction, i.e., withdrawal; and
the last section centers on the perplexing problem of directional hearing in
sharks.

The Species List for Attraction

All species of sharks that have been examined in the field (Table 1) have
been found to be attracted to specific types of synthesized sounds as well as

Table 1. Summary of experiments in which sharks were attracted to an underwater transducer (speaker) during playback of low-frequency, pulsed sounds.*

Family and species	Common name	Sound†	Author(s)
Alopidae			
Alopias sp.	Thresher	HF (N)	Nelson & Johnson (unpublished)
Carcharhinidae			
Carcharhinus sp.		FN (A)	Nelson & Gruber 1963
		FN (A)	Richard 1968
		FN, SqW (A)	Myrberg et al. 1969
C. albimarginatus	Silvertip	FN (A)	Nelson & Johnson 1972
C. falciformis	Silky	FN (A)	Nelson et al 1969
		SpF (N)	Evans & Gilbert 1971
		FN (A)	Myrberg et al. 1972
			Myrberg et al. 1975a
			Myrberg et al. 1975b
			Myrberg et al. 1976
C. leucas	Bull	FN (A)	Nelson & Gruber 1963
C. longimanus	Oceanic whitetip	FN (A)	Myrberg et al. 1975a
			Myrberg et al. 1975b
			Myrberg et al. 1976
C. melanopterus	Blacktip reef	FN (A)	Nelson & Johnson 1970
		FN (A)	Nelson & Johnson 1972
C. menisorrah	Gray reef	SpF (N)	Brown 1968
		FN (A)	Nelson & Johnson 1970
		FN (A)	Nelson & Johnson 1972
C. springeri	Reef	FN, SqW (A)	Myrberg et al. 1969
Galeocerdo cuvieri	Tiger	FN (A)	Nelson & Gruber 1963.
Negaprion brevirostris	Lemon	FN (A)	Nelson & Gruber 1963
		BbN (A)	Banner 1968
		FS (N)	Banner 1972
Negaprion fosteri	"Lemon"	FN (A)	Nelson & Johnson 1972
Prionace glauca	Blue	HF, StF (N)	Nelson & Johnson
		FN (A)	(unpublished)
Rhizoprionodon porosus	Sharpnose	FN (A)	Richard 1968
		FN, SqW (A)	Myrberg et al. 1969
Triaenodon obesus	Reef whitetip	SpF (N)	Brown 1968
		SpF, StF (N)	
		FN (A)	Nelson & Johnson 1970
		FN (A)	Nelson & Johnson 1972
Lamnidae			
Isurus oxyrinchus	Mako	HF, StF (N)	Nelson & Johnson (unpublished)
Orectolobidae			
Ginglymostoma cirratum	Nurse	FN (A)	Richard 1968
		FN, SqW (A)	Myrberg et al. 1969
		FN (A)	Nelson et al. 1969
Sphyrnidae			
Sphyrna sp.	Hammerhead	FN (A)	Nelson & Gruber 1963
S. tiburo	Bonnethead	FN (A)	Nelson et al. 1969

*Taken in part from Nelson and Johnson 1972.
†Types of artificially produced (A), and naturally recorded (N) pulsed sounds: FN, filtered random or white noise; BbN, broadband noise; SqW, square waves; SpF, speared struggling fish; HF, hooked struggling fish; StF, stampeded group of fish; and FS, fish sounds.

to a variety of biological sounds. The group includes species commonly found over shallow flats and reefs as well as over deep oceanic waters. Most are piscivorous, but the group also includes a number of small species that feed mainly on certain invertebrates (e.g., the bonnethead, *Sphyrna tiburo*). The attractiveness of such sounds to sharks of such diverse habits suggests strongly that most, if not all, other carnivorous sharks will react similarly to appropriate underwater sound sources. It is noteworthy that other carnivorous fishes, such as groupers and snappers, find the same types of underwater sounds attractive, although their approach to the sources is much slower than that of sharks (Myrberg et al. 1969, Nelson and Johnson 1970, Nelson et al. 1969, Richard 1968, Steinberg et al. 1965).

Qualities of Attractive Sound

Early experiments showed that not all sounds elicit approach in sharks. This suggested that such animals are attending to specific qualities of transmissions. To determine these qualities, we synthesized sounds to control systematically those features of obvious interest. By this means alone or with natural and synthesized sound used together in the same experimental design, findings from the field related closely to the meager but significant results previously obtained on the hearing physiology of some sharks.

Spectral Content—One important feature of an attractive sound was its spectral content. All results indicated that a sound, to be attractive to sharks, must contain frequencies below 800 or 1000 Hz; if not, approach was not seen (e.g., Myrberg et al. 1969); see Figure 1. The initial findings by Nelson and Gruber (1963) suggested that only very low frequencies were attractive, i.e. below 60 Hz. This figure was revised upwards, however, after subsequent studies showed that signals possessing higher frequencies were also attractive (Myberg et al. 1972, Nelson and Johnson 1972). Yet, for those species most intensively studied (e.g., the silky shark, *Carcharhinus falciformis*), levels of attraction increased as the included wavelengths of a signal increased (Myrberg et al. 1972); see Table 2. This cline of responsiveness eventually ended in similar effectiveness when octave bands of very low frequencies were finally reached, i.e., 10 to 20 Hz and 20 to 40 Hz (Myrberg et al. 1975a, 1976). All synthesized sounds used during controlled testing, however, were bands of limited frequencies, and experiments have not yet determined the band limits of an attractive sound. Nevertheless, doubtless any signal whose spectrum includes an octave or more and contains frequencies below 800 Hz will be attractive—so long as it possesses a few additional qualities.

The upper frequency limit of attractive sound, around 800 to 1000 Hz, agrees well with data on the hearing abilities of those few species of sharks tested under reasonably controlled conditions (the lemon shark, *Negaprion brevirostris*—Banner 1967, Nelson 1967, Wisby et al. 1964; the bull shark, *C. leucas*—Kritzler and Wood 1961; the scalloped hammerhead, *S. lewini*—Olla 1962). It is noteworthy that this limit is extremely close to the upper limiting frequencies that elicit vestibular microphonics in another elasmo-
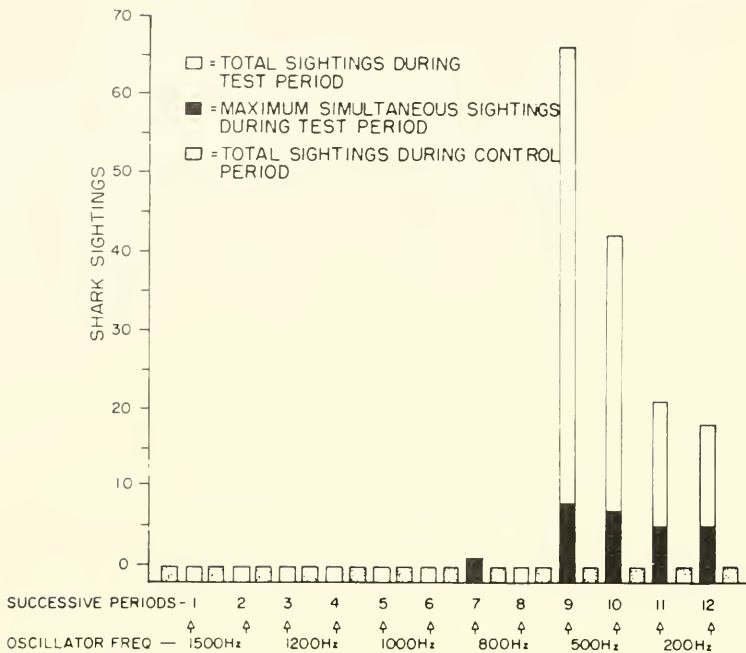
Figure 1 Attraction of sharpnose sharks *Rhizoprionodon*, sp., by acoustic signals, showing upper effective frequency limit. Signals consisted of irregularly pulsed, overdriven sine waves, having fundamental frequencies from 200 to 1500 Hz. Peak sound pressure level at 18.5 m from sound source was approximately 20 dB above broadband ambient noise. Each test and control period—3 min. (Myrberg et al. 1969)

branch, the skate, *Raja clavata*. Also, the most effective frequency range for purposes of attraction, between 10 and 100 Hz, approximates the range of frequencies associated with spike discharges from vibration-sensitive areas of the labyrinth in this skate (Lowenstein and Roberts 1951). Although the authors attached no physiological significance to their data, that their findings correlate with those of others at a different level of integration indicates a functional significance between these neural and behavioral events. Additional examination of hearing ability and its neural correlates in other elasmobranch fishes will certainly provide further insight into such a suggestion.

Pure tones, regardless of frequency, do not attract free-ranging sharks (Myrberg et al. 1969, Richard 1968). The scarcity of biologically produced pure tones in the aquatic environment may explain this failure to react, thus reinforcing the belief that the attraction response to low-frequency, broadband sounds has a biologically adaptive basis.

Repetitive Pulsing—The second quality of an attractive sound is its repetitive pulsing. Continuous sound, regardless of frequency, does not elicit

Table 2. Differential attraction of silky sharks by irregularly pulsed, instrumental signals of different frequencies (Straits of Florida).*

Session	Frequency band of signal (Hz)	No. of sessions	Surface area covered by signal (S/N=0)	Surface area covered by signal (S/N=20)	Total no. of sharks attracted	Actual no. of sharks per session	Relative level of attractiveness (predicted no.† ÷ actual no.)
Test	25–50	85	0.036 km²	0.0018 km²	29	0.34	918.9
	75–150	85	1.044 km²	0.046 km²	13	0.15	16.1
	250–500	85	43.32 km²	0.81 km²	20	0.24	1.5
	500–1000	85	36.75 km²	0.69 km²	12	0.14	0
Control	No signal	177	—	—	12	0.007	—

*Modified from Myberg et al. 1972.

†If all signals were as attractive as the 500-1000 Hz signal (areas arbitrarily chosen are those having S/N = 20).

attraction (Hobson 1963, Nelson and Gruber 1963, Wisby et al. 1964, personal observation). Banner (1972) demonstrated in young lemon sharks that the rapidity of pulsing is directly related to the relative attractiveness of various biological sounds produced by their prey. This same correlation existed in silky sharks for synthesized sounds so long as their spectral content was appropriate (Myrberg et al. 1972). Although extremely low frequency signals cannot be pulsed rapidly and still maintain their spectral integrity, even a constant pulse rate of 1/s appears to be slightly attractive (Myrberg et al. 1972) (Table 3).

Since natural sounds often show irregularity in their pulse structures (e.g., erratic movements during feeding, flight, and stress), attention to this type of signal should be highly adaptive to any predator. Accordingly, it is not surprising that the most attractive sounds have irregular pulses (Myrberg et al. 1972, Nelson and Johnson 1972). Only two studies have compared the effects of natural and synthesized sounds. Nelson and Johnson (1970) believed that the sound produced by a "stampeded" school of bonefish (*Albula vulpes*) elicited slightly stronger response from their subjects than similarly structured synthesized sounds. In contrast, Banner (1972) showed that a synthesized sound possessing characteristics most conducive to attraction (i.e., rapid pulsing, low frequency, sufficient loudness) was generally as effective as natural sounds.

Unfortunately, we know little about the degree to which sharks recognize differences between extremely brief intervals within given sounds. Yet, there is no a priori reason why their ability should be less than the recognition afforded remarkably small intervals (less than 10 ms) by various teleosts

Table 3. Differential attraction of silky sharks to various instrumental signals, each having a different pulse character; all signals had the frequency spectrum of 25–50 Hz (Tongue of the Ocean, Bahamas).*

Sessions	Pulse nature of signal	No. of 3-min periods	No. of sightings of sharks[†]	Sightings per period ($\bar{x}$)
13 test sessions	Irregular	13	33	2.6
	10 Hz	13	24	1.8
	5 Hz	13	17	1.3
	1 Hz	13	14	1.0
13 control sessions	No signal	65	34	0.5
	Total		122	

*From Myrberg et al. 1972.
[†]Distribution of sightings among the signals is significantly different from random distribution: –0.05 (Kolmogrov-Smirnoff, one sample test).

that have been recently examined (Ha 1973, Myrberg et al. in press, Spanier 1975). Probably temporal processing by the acoustical modality of fishes is an extremely sensitive and precise function.

Another important finding by Banner (1972) was that young lemon sharks do not respond to the specific nature of the source, i.e., prey versus nonprey; instead, differentiation rests upon the temporal characteristics of the sound, this being even more important than frequency composition. A similar conclusion was reached by others dealing with adult sharks (Myrberg et al. 1975, Richard 1968). Thus, this could not be attributed simply to the apparent inexperience of Banner's subjects. Since such sounds are commonly produced by prey (Banner, 1968, Hashimoto and Maniwa 1967, Moulton 1960, Nelson and Johnson 1970), the findings suggest that the rapid investigation of certain types of sounds results in enough opportunities for prey capture that appropriate responsiveness remains despite the fact that various types of nonprey sources produce similar sounds. Also, the effects of habituation on responsiveness in the absence of positive reinforcement indicate that energy would not be wasted for long in response to sounds produced by sources other than prey (see page 404).

Nelson and Johnson (1972) examined variation in pulse rate on acoustic attraction in four species of Pacific reef sharks (Fig. 2, Table 4). They found that low-frequency, pulsed sounds were more attractive if they had irregular rather than regular pulse intervals. No significant difference was found, however, between trains of pulses having equal duration and those having variable duration.

Sound Level—To be effective, a sound must be loud enough that subjects can hear it and orient to it. This factor is realistic only when ambient noise level is considered at the time of stimulation. The auditory
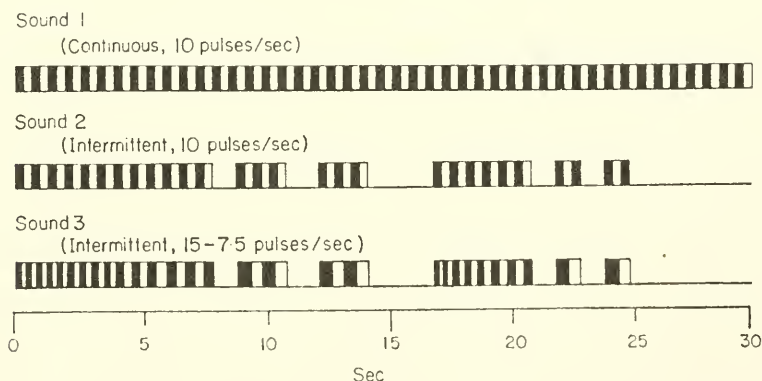
Figure 2 Diagrammatic representation of three 25–to 100–Hz pulsed sounds used for playback to sharks. The vertical black and white bars represent pulses (bursts of noise) but are not drawn to the absolute time scale. The 30-s sequences illustrated were repeated 10 times to comprise single 5-min playback periods. (Nelson and Johnson 1972)

Table 4. Number of sharks sighted and mean response intensities for three 25- to 100-Hz pulsed sounds. Based on 15 sound and 15 control periods for each sound (Eniwetok).*

Frequency	Number of sharks		Response intensity
	Sound	Control	
Sound 1, 10 Hz continuous	51	12	3.83
Sound 2, 10 Hz intermittent	102	18	5.25
Sound 3, 15 to 7.5 Hz, intermittent	100	14	5.50
Total	253	44	4.86[†]

*From Nelson and Johnson 1972.
[†]Mean of the maximum observed response intensities for each sound period; values based on an arbitrary eight-point scale.

sensitivity of fishes appears to be affected not only by prevailing ambient levels (Banner 1972, Buerkle 1968, Ha 1968, Tavolga 1967) but also by high ambient levels experienced prior to tesing (Ha 1968, Popper and Clarke 1976). Many workers have nevertheless neglected ambient noise as a potential source of disturbance. When responsiveness has been considered in sharks and teleosts, it often ceases when the signal level drops to a point between 15 and 25 dB above the prevailing spectrum-level ambient (e.g., Banner 1967, 1972; Buerkle 1969; Cahn et al. 1969; Myrberg et al. 1969; Nelson 1967). Thus, although a given signal may propagate quite far through the medium before falling below the spectrum-level noise, it probably reaches either an inaudible level or a level inadequate for response long before that distance is attained.

This illustrates one major difference between natural biological sounds and the synthesized signals frequently used in field studies of shark attraction. Although such studies occurred where sharks were rare or variable daily, an adequate sample size was, nevertheless, essential for meaningful analyses. This requirement thus forced signal transmissions to be at levels sufficient to provide reasonable areas of coverage. Such transmissions often attained levels of between +37 and +55 dB/μbar re 1 m. With a relatively smooth sea, such signals could reach many hundreds of meters from the sources before reaching ambient levels. Only a few biological sounds reach these rather high levels. Therefore, one can predict that most biological sounds of interest to sharks probably are detected only at distances much less than 100 m from the source.

An extreme example may be the maximum detection distances that Banner (1967) found for various prey sounds, using young lemon sharks in very shallow water ($\sim$30 cm depth). Such detection distances never exceeded

4.5 m for the prey sounds or 10 m for various synthesized sounds. These extremely short distances were understandable in this case because of the nature of the prey sounds used and the extreme attenuation of signal strength due to the extremely shallow water. These conditions allowed Banner to demonstrate, however, that the sharks responded by well-oriented approaches toward the sound source only when they were in an area in which the sound level exceeded that of previously established hearing thresholds of similar-sized animals under controlled laboratory conditions (see Fig. 3—measured in displacement values based on demonstrated sensitivity (Banner 1967)). Thus, at least in this case, directional responses appeared at the greatest detection distances. This differs somewhat from results of Chapman and Johnstone (1974) for the cod, in which the level required for directional responses in members of that species was higher than that necessary for detection. One reason for this difference may be the presence of a swim-bladder in the cod and its absence in sharks.

Qualities of a Sound that Appear Unimportant for Attraction

Within reasonable limits, certain features of underwater sounds are apparently not critical for attracting sharks. These include the duration of individual pulses within a given train of pulses (Nelson and Johnson 1972) and the summation, in time, of acoustic energy present in part or all of the pulse train (Myrberg et al. 1972). The latter study showed that silky sharks apparently find a signal with a train of 20 pulses/s, each pulse lasting 10 ms (i.e., 200 ms of energy), far more attractive than a signal of only 10 pulses/s, each pulse being 50 ms (i.e., 500 ms of energy). The latter, however, was far
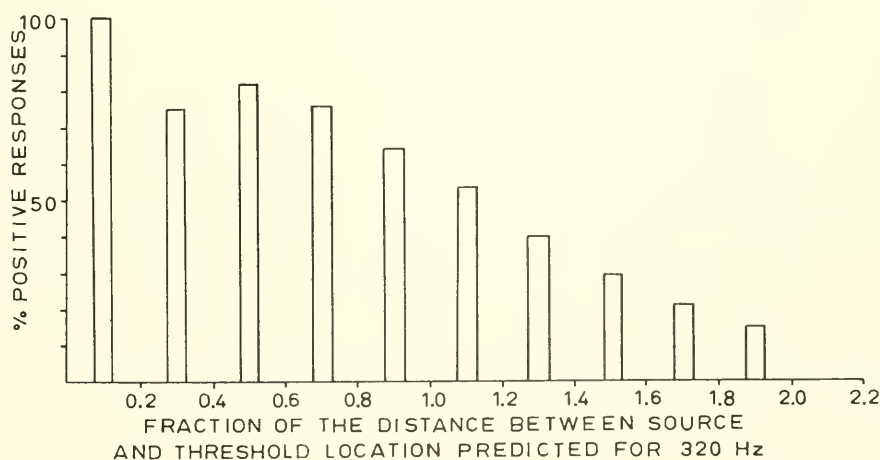
Figure 3   Percentages of positive responses by young lemon sharks at locations within and beyond the predicted threshold distance, the latter being calculated from displacement thresholds as measured in the laboratory for conspecifics of the same size. Results of attractive signals having optimum level at 320 Hz are combined. (Banner 1972)

more attractive than a third signal with 5 pulses/s, but whose pulses were also 50 ms (i.e., 250 ms of energy).

Behavior of Sharks Subsequent to Attraction

As an ethologist, I am pleased that attraction studies have provided knowledge in areas other than that concerned simply with the acoustical factors underlying attraction per se. Acoustic attraction has provided an excellent background for studying the behavioral activities of various species of sharks in their natural environments; in most cases, the only environment where that is possible, based on present inability to maintain the animals under conditions appropriate for their health or for observation (Gruber and Myrberg 1977; Myrberg, 1976). Although each field observation may be brief, the knowledge gained over many periods can aid, for example, in placing appropriate laboratory findings in reasonable perspective (Banner 1972, Evans and Gilbert 1971). Acoustic attraction also reduces dependence on fortuitous encounters with sharks or on situations that often involve intense feeding activities (e.g., when food is used to attract animals). Such situations often result in rapid movements by sharks and preclude either the observation or the occurrence of numerous behavior patterns exhibited under less highly motivated conditions.

Although the behavioral activities noted during sonic attraction must be associated with that context, such activities often relate to other contexts. For example, the final approach to a loudspeaker may result in sharks striking, biting, and even swallowing the entire apparatus (Banner 1968, Myrberg et al. 1969, Nelson and Johnson 1972). This clearly suggests that such animals need not perceive chemically or visually a familiar (food?) object before attacking. This contrasts with earlier claims that complete feeding patterns are elicited only in the presence of complimentary stimuli involving different sensory modalities. Also, specific motor patterns that have been performed by various species of sharks during encounters with man, e.g., "hunching" (Johnson and Nelson 1973, Myrberg and Gruber 1974), have been noted several times directly in front of a transducer at the end of a rapid approach (Nelson and Johnson 1972).

These quite differing contexts, as well as others with similar patterns, appear to possess one common feature—the condition favoring conflict between the tendencies of approach and withdrawal. The apparent result is hesitance in carrying out either activity. This suggests that "hunching" may be an expression movement rather than a ritualized social display that is released only by a specific stimulus (configuration). These few examples point out how observations may lead either to better understanding of the probable reasons for the occurrence of specific activities or, at least, to predicting and possibly even controlling the behavior of these predators.

Many species-typical action patterns by a variety of sharks have been observed in the vicinity of a sound source. Many of these patterns have been described by Myrberg and Gruber (1974); the rest are explained in the actual accounts (Banner 1968, 1972; Myrberg et al. 1969, 1972, 1975a; Nelson and

Johnson 1970, 1972). The list of patterns includes those performed in apparent social contexts as well as others directed at the transducers themselves. The first group includes parallel swimming, circling, leaning, following, chasing, giving way, and hunching; the patterns of the second group include circling the transducer, veering off, hunching, biting, "startle," and head shaking. Patterns observed in either of these specific contexts include spinning, gill puffing, yawning, head shaking, and thrusting.

Given the same context, the strength of response appears to vary considerably among sharks (Limbaugh 1963). This has been shown clearly during sonic attraction. Nelson and Johnson (1972) found that the gray reef shark (*C. menisorrah*) approached an operating transducer more rapidly and more closely than did the reef whitetip (*Triaenodon obesus*).

The ultimate intensity of movements by sharks in a restricted area is often considered to be the feeding frenzy. Undoubtedly, this phenomenon is initiated and maintained partly by the social facilitation of movement by active sharks being close to one another. Such an effect has been noted in the vicinity of an active transducer that obviously provided an adequate stimulus situation. Relative speed of movement (Myrberg et al. 1969), intensity of approach (Nelson and Johnson 1972), and competitive feeding (Nelson et al. 1969) increase as sharks concentrate around a sound source. This suggests that the underlying motivation controlling the appropriate patterns of movement changes along a continuum and thus allows the energy used in specific activities to be adjusted to some level of apparent competition. Also, the number of sharks attracted to a source appears related to the number of sharks present within hearing range of the sound, at least during early trials (Banner 1972, Myrberg et al. 1975a, Nelson and Johnson 1972). This implies that attraction is effected regardless of motivational differences. Possibly a single common motivation underlies such responsiveness, but this is speculative. There is no a priori reason to believe, however, that motivation cannot change once a subject is close to the source.

Although hundreds of tests have been conducted on shark attraction there is a notable absence of reports on either intra- or interspecific aggression involving these animals, even when many are moving about in a relatively small area. This lack of obvious aggression has also been reported in various laboratory contexts (Myrberg and Gruber 1974). Yet at least some species—and probably all—possess a social organization apparently based on a dominant—subordinate system (Allee and Dickinson 1954, Clark 1963, Myrberg and Gruber 1974). Such an organization also crosses species lines (Cousteau and Cousteau 1970, Limbaugh 1963, Springer 1963, 1967). Although dominant—subordinate systems have been traditionally described and discussed within the context of aggression in other animal groups, the system as presently described in sharks might involve quite another context, i.e., predator—prey, the larger individual or the group being the potential predator and the smaller individual or the single animal within the group being the potential prey. Although there is some evidence mitigating against this idea (e.g., sexual differences—Myrberg and Gruber 1974), if that relationship is actually the basis for the apparent hierarchical organization in sharks, the continued use

of terms such as dominants and subordinates may be as relevant as applying such terms to cats and mice or foxes and rabbits. Future research will, it is hoped, bring added understanding to this problem.

The predatory nature of sharks relates well to their rapid response to sounds of wounded, struggling fishes. Yet, predation must surely extend beyond that limited source of food (Hobson 1963, Nelson et al. 1969). Banner (1968) hypothesized that hydroacoustic stimuli associated with normal feeding behavior in other fishes may also stimulate sharks to feed. This could be an even more significant stimulus than that from struggling prey. Such reasoning could explain why sharks are attracted to the natural sounds of prey and nonprey alike (Banner 1972). This leads to the inevitable conclusion that learning should play a role in the attraction process. Sharks have amply demonstrated not only that they are able to learn rapidly and retain a wide variety of tasks (e.g., Aronson et al. 1967, Graeber 1972, Graeber and Ebbesson 1972, Gruber and Schneiderman 1975) but also that individuals of different ages (based on size) within a given species often react quite differently in the same situation. For example, small members of many species are invariably more "curious," more "nervous," more unpredictable in their movements, and far less cautious than that noted in larger individuals (personal observation). This difference could be due to ontogenetic changes uninfluenced by experience, but under the conditions of relative food scarcity encountered by many, if not all, species of sharks, it would seem that the evolution of such carnivorous predators could ill afford food-related activities to be totally uninfluenced by experience.

One learning process commonly exhibited by sharks in the field is habituation to an attractive sound when trials are massed over a short period in the absence of reinforcement (Myrberg et al. 1969, Nelson et al. 1969) (Fig. 4). Nelson and Johnson (1972) suggested that this process might have affected daily variations in the results of their study. Myrberg et al. (1969) found, however, that at least in sharpnose sharks (*Rhizoprionodon* sp.—probably *R. porosus*), prehabituation levels of response to sounds reappeared approximately 1 hr after all sound transmission had stopped.

Such a learning process is apparent in other contexts as well. For example, Nelson and Johnson (1970) reported the following case: an 11-kg (25 lb) grouper had been speared but escaped deep into a hole where neither divers nor sharks could reach it. Within minutes, odors and sounds had attracted several reef whitetips that excitedly circled the site and explored the various holes leading to the fish. The sharks were unable to reach the wounded animal, and their excitement soon waned. Shortly, all disappeared. Chumming with fresh bait for the next hour at the site failed to attract a single shark. The probable importance of "learning to ignore" in these animals should not be regarded lightly by researchers, lest their hard-won data contain a sizable artifact.

The Antithesis of Approach—Withdrawal

An interesting and apparently paradoxical effect on the behavior of the few species of sharks examined thus far concerns the elicitation of a response
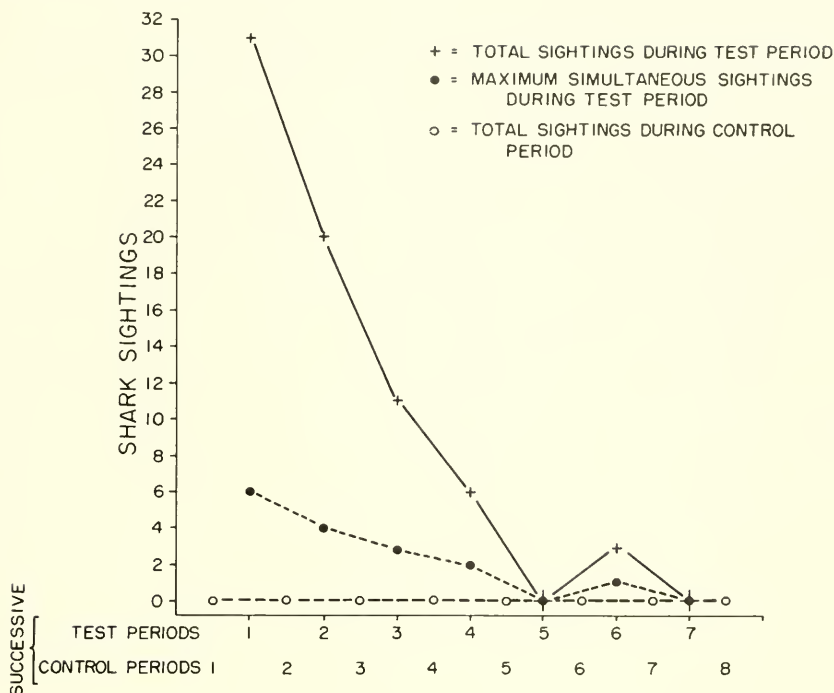
Figure 4 Decrease in sightings of sharpnose sharks, *Rhizoprionodon* sp., through successive test periods. Signals consisted of constant level, irregularly pulsed, overdriven 80 Hz sine waves (biphasic, symmetrical, and distorted square waves). Each test and control period—3 min. (Myrberg et al. 1969)

opposite to that of rapid approach, i.e., withdrawal—elicited by the use of sound. This unique pattern of response was reported by Banner (1972) during his bioacoustical study of young lemon sharks. His close attention to their behavior provided coherent clues as to probable causal relationships. Rapid flight appeared to occur at the precise moment certain sounds began. This was particularly true if sharks were approaching the source. Banner noted, however, that sounds consisting of closely spaced pulses rarely caused such responses. He thus hypothesized that during rapid approach, short intervals allowed the signal level to increase smoothly, which would not occur when a loud, impulsive sound, such as a single pulse or a signal possessing long intervals (seconds in length) was projected. Long intervals resulted in sudden jumps in level being experienced during an approach response. Only these two sound types, interestingly, elicited the so-called "startle" response in this study. As in the case of attraction, Banner found that these "startle" responses occurred only when the projected sound level at the location of "startle" was well above the established threshold sensitivity.

  Strikingly similar withdrawal responses were first observed in silky sharks by our team in 1970 during a field study in the Tongue of the Ocean, Bahamas. Water depth was approximately 2000 m at the test site, and the

sharks were adults or subadults. These responses indicated that the withdrawal responses noted by Banner (1972) were not due to the shallow depth at his test site or to his young subjects.

Two years later we began to explore this phenomenon more fully with the idea that a successful predator, when approaching a source transmitting signals having appropriate acoustical properties, might well change its behavior when the properties to which it was attending suddenly changed in an "unexpected" manner. Since predatory mechanisms, behavior or otherwise, obviously require energy expenditure, an appropriate and highly adaptive mechanism for any predator would be one that would aid "go-no go" decisions regarding problems involving conservation of energy in such contexts. If such a mechanism were operating, sudden changes in the expected flow of information being processed at the moment could rapidly influence ongoing behavior.

The results of the ensuing studies will appear elsewhere, but a brief summary follows. It became clear that rapid withdrawal could be elicited in adult lemon and silky sharks by projecting underwater sounds similar to those eliciting approach in the first place. The frequency spectrum was the same in both types of sounds (i.e., broadband, covering one or more octaves within the hearing range). Also, both types could be pulsed. There were differences, however; sounds eliciting withdrawal possessed rather long intervals (e.g., 2.5 s on, 2.5 s off). This distinction paralleled that noted by Banner (1972).

An additional factor, mentioned by Banner as perhaps playing a role in the response, appeared to be most important. This concerned a sudden increase in the sound level as a subject approached the given source. For example, a sudden increase of 15 to 20 dB when a shark was within a few meters of the source resulted in the animal's retreating at a speed often faster than its initial approach. This response has been frequently documented from captive adult lemon sharks, held under conditions adequate for appropriate testing (Klimley 1976), as well as by young and adult free-ranging silky sharks found in the offshore waters of the Straits of Florida and the Tongue of the Ocean, Bahamas (Myrberg et al. 1975b). The basic response pattern was similar in both species; the restrictions imposed on the movements of the lemon sharks by the test facility (a large channel) resulted, however, in a more stereotyped form of withdrawal. Basically, this consisted of the shark moving in a reasonably narrow arc and proceeding in the direction from which it came. The pattern shown by silky sharks was more variable—probably due to the lack of any restrictions on their movements. The general response was neither a simple "startle" nor a series of rapid turns or sequences of apparently disorganized movements; rather, it consisted of a sequence of movements resulting in the animal(s) disappearing from view within 10 to 30 s of the change in the ongoing sound.

The orientation of such movements also showed that the animals recognized the direction of the sound source. Some individuals turned within a second or two of signal change through a narrow arc and headed out of visual range; others that had been heading directly at the transducer veered

off to the right or left and also headed out of view. These responses usually occurred when sharks were within 5 m of the source. Sharks farther from the source generally moved in a wide arc shortly after signal change, often disappearing from view in the direction from which they came. Although signal change was initiated when one shark had reached a distance of 5 to 10 m from the source, sharks farther away also reacted accordingly.

These sequences were especially clear for silky sharks approaching the transducer during withdrawal tests in the Straits of Florida. Silky sharks tested at specific locations in the Tongue of the Ocean exhibited greater variation in response. In the Straits, variations from the relatively rapid drift of our vessel during a given day of testing, the different drift tracks used each day, and the relatively few sharks seen on a given day substantially reduced any possible effects of habituation. In the Tongue of the Ocean, the same small population of silky sharks congregating around an offshore, deep-moored buoy was tested over a period of some hours. This provided the opportunity to determine possible changes in levels of responsiveness during repetitive testing. These data indicated that the "intensity" of withdrawal wanes if trials follow one another frequently. For example, if an appropriate sound is transmitted once every 5 to 10 min, rapid withdrawal is seen during the first two or three trials; less rapid withdrawal is elicited during the next few transmissions; finally, if the same sharks are attracted again within a few minutes, there will probably be little or no change in behavior when the supposed aversive stimulus is transmitted. Observers who have monitored both approach and withdrawal feel that withdrawal is more resistant to the effects of habituation, but this must be evaluated more precisely.

Clearly there are species differences in withdrawal response. Initial tests in the Tongue of the Ocean failed to bring about withdrawal in a small number of oceanic whitetip sharks (*C. longimanus*) under conditions that resulted in excellent withdrawal by silky sharks of similar or larger size. Although a more precisely monitored recent experiment showed that such responses could be elicited consistently at least two or three times by whitetips under nonfeeding conditions, much work remains before enough knowledge is gained to explain these species differences.

Finally, pure tones appeared quite ineffective in eliciting withdrawal, even at high levels. This fits closely the fact that such sounds are also not attractive to free-ranging sharks. This lack of response certainly implies an inability by these animals to hear pure tones, but this is not true based on laboratory findings using appropriate training techniques. This must mean that in the natural environment such tones have little or no meaning.

Various workers have tried to elicit withdrawal or avoidance responses from teleosts or, at least, to redirect their movements by using sounds. While a few have had varying degrees of success (e.g., Chapman 1976, Shiskova 1958, van Derwalker 1967), many have failed (e.g., Burner and Moore 1953, Miyake 1952, Moore and Newman 1956). Most reports mention initial startle responses to high-level, low-frequency sounds, but subjects apparently adjust rapidly to such sounds and return within moments to prior levels of activity. This again implies that such sounds have little aversive significance

for fishes, regardless of group. It may also mean, however, that the test animals were able to assess rapidly the relative value of specific stimuli in specific contexts. Thus, under the artificial conditions often present in "biological engineering" designs, fishes may well show no adaptive response because the context neither calls for it nor requires it. An appropriate context involving sounds of predators may well bring about unmistakable avoidance or withdrawal responses (e.g., Moulton 1960, Steinberg et al. 1965). Further study will surely expand on this point, but our findings with silky and lemon sharks suggest that appropriate sounds may indeed be aversive in the biologically adequate context.

Other workers mention similar withdrawal responses by sharks confronted by sudden sounds, such as yelling underwater (Eibl-Eibesfeldt and Hass 1959). Yet, there are also reports to the contrary (e.g., Hobson 1963). Although these differences may have been due to species differences or to variations in underlying motivation, possibly the latter case either involved sound levels below the hearing thresholds of the oncoming sharks or they did not cause a sufficient change in ongoing events to cause the animal to change course.

Apparently sound level per se cannot be the entire answer, since one sound at a given level will result in rapid withdrawal by sharks, while another at that same or even slightly higher level will attract them right up to the source. The real key to the problem may actually be the progressive increase in loudness as perceived by an approaching shark. Attraction may be initiated and maintained by moving toward a given sound whose level increases smoothly (relative to some unknown reference). Withdrawal, on the other hand, may be initiated and maintained by a sound whose structure results in sudden, increased levels, these levels differing greatly (relative to that same reference) from that just previously experienced (or expected) during approach. This implies that the shark is aware of a normal increase in level as it approaches the source. This formulation can be fitted into the well-known Biphasic Theory of approach/withdrawal processes as proposed by Schneirla (1959, 1965). Although the theory had originally been used to explain the organization of processes underlying early behavioral development in vertebrates, its author and others have subsequently attempted to apply it to other stages as well. The theory maintains that intensity of stimulation determines the operative process, i.e., approach or withdrawal. Undoubtedly, intensity plays an important role in attraction and withdrawal behavior in sharks but that factor per se seems less important than the nature of the increase in intensity during approach. The apparent importance for sharks of the latter factor suggests that its role should be evaluated in other groups of animals.

Directional Hearing

The rapid and directed orientation of free-ranging sharks to distant sound sources had been, until recently, the single exception to certain "rules" formulated by van Bergeijk (1964, 1967) in his popular theory of acoustic

orientation in fishes. In that theory, he maintained that (1) orientation to a sound source occurs only when a fish is extremely close to that source (i.e., within the acoustic near-field (see page 392) and (2) oriented responses are mediated only through the lateral line system, the labyrinths being precluded from that function.

This theory was of concern to naturalists and other functional biologists interested in the adaptive value of sound detection in fishes. Although it was clear that sharks and teleosts could detect low-frequency sounds, serious questions could be raised as to the importance of any such detection when sound sources had to be localized through random movement. Initial studies indicated that fishes followed the rules, the single exception being the sharks. Early tests of acoustic orientation in teleosts were invariably carried out under the acoustically complex conditions of the laboratory setting (for a critique, see Chapman and Hawkins 1973, Sand and Enger 1974). The only studies conducted in open waters were those centering on sharks, and these went counter to the rules. This suggested that some important factor or factors had been overlooked by van Bergeijk and subsequently by the adherents of his theory. If so, at least some teleosts might well also demonstrate far-field orientation under appropriate conditions.

These conditions were recently provided at various locations, with experiments being carried out at sea (Chapman 1973, Olsen 1969 in Sand and Enger 1974, Sand and Enger 1974, Schuijf 1974, Schuijf and Siemelink 1974, Schuijf et al. 1972) as well as in the laboratory (the latter using electrophysiological techniques—Enger et al. 1973, Sand 1974). These studies showed that selected teleosts can orient to sound in the far-field and in a number of instances that orientation is effected through the labyrinth organs.

Interest in spatial orientation has now shifted to the fascinating problem of its precise underlying mechanisms. Recently, Schuijf (1974) has proposed an elegant model to explain directional hearing in sharks and in teleosts without swimbladders. First, it states that directional hearing is not limited by distance, so long as the signal level exceeds the threshold for directional hearing at the existing noise level. Also, such animals can localize a sound source only if, in addition to the radial component of the particle displacement at the position of the fish, there is also a tangential component in the vertical plane through the source and the fish's position. The importance of the vertical plane to this model corresponds well with evidence that fishes are sensitive to displacements in both the horizontal and vertical planes.

The carefully reasoned theory certainly can explain instantaneous resolution of the 180° ambiguity within certain depth restraints. Once that ambiguity is resolved, even slight head movements of a moving shark should provide accurate information relative to a right-left decision if there is adequate detection by both ears and the respective axes of maximum sensitivity within the appropriate maculae are not parallel (see Vilstrup 1951, Figs. 16, 17, and 18). Differences in the amplitude of the microphonic potentials from the two labyrinths of the perch, *Perca fluviatilis*, have been shown to depend on the direction of vibration (Sand 1974; see discussion in Sand and Enger, 1974).

Another highly intriguing feature of hearing in sharks concerns those areas in the labyrinth that supply the information necessary for their demonstrated sensitivities and orienting abilities. Lowenstein and Roberts' (1951) study of the ear of the skate, *Raja clavata*, was for many years the only electrophysiological study of the elasmobranch labyrinth. Their findings demonstrated that low-frequency vibrations were adequate to elicit propagated discharges from the anterior portion of the saccular macula, a portion of the utricular macula (*lacinia utriculi*), and the macula neglecta. Vilstrup (1951), using lesion techniques, found acoustical sensitivity, however, only in the pars inferior of the spiny dogfish, *Squalus acanthias*. This was the state of the knowledge until only recently, when the macula neglecta, a little-known structure near the dorso-posterior aspect of the sacculus (Figure 5) came under study by Tester et al. (1972) and Fay et al. (1974). Because of its relative position and its high sensitivity to frequencies within the hearing range of sharks, it probably does detect sound. Electrophysiological experiments by Fay et al. (1974) on the blacktip reef shark, *C. melanopterus*, showed that the largest microphonic responses obtained from the macula neglecta
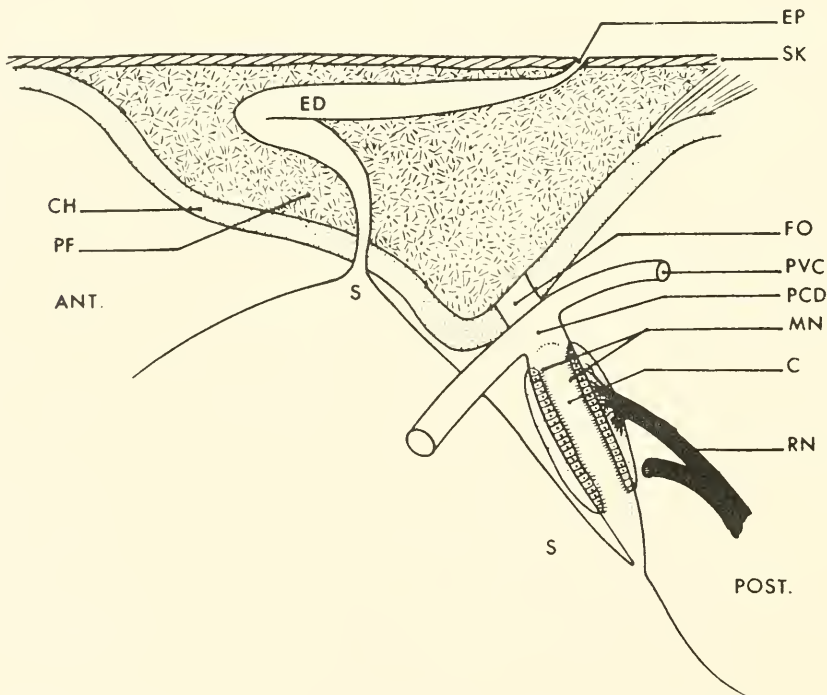
Figure 5 Schematic section of portions of the ear of the blacktip reef shark, *Carcharhinus melanopterus*. C. Cupula; CH, chondocranium; ED, endolymphatic duct; EP, endolymphatic pore; FO, fenestra ovalis; MN, macula neglecta; PCD, posterior canal duct; PVC, posterior vertical canal; PF, parietal fossa; RN, ramus neglectus nerve; S, sacculus; SK, skin covering fossa. (Fay et al. 1974)

were recorded when the parietal fossa was stimulated by slight vibrations, the response level falling rapidly as the stimulus was moved away from that location. They also found that the levels of response depended on the location of stimulation within the surface confines of the fossa. These findings, plus the remarkable similarity of various structures of the region (i.e., parietal fossa, the taut membranes of the fenestrum ovalis, the macula neglecta, and the latter's proximity to the sacculus and its associated endolymphatic duct) to the tympanic membrane and its associated structures in higher vertebrates cause one to question if this is merely coincidence. The obvious question is: why would a shark possess a tympanic-like membrane in its auditory system? Could the structure act like the swimbladder of teleosts, which transforms slight pressure fluctuations into appropriate displacements so as to increase sensitivity to far-field sound? The main difficulty is the absence of an apparent impedance discontinuity in the system. Could it be the seat of a nondirectional reference for the timing analysis, as required by Schuijf's model of directional hearing? The data strongly suggest that the maculae neglecta, lying directly below the membranes of the fenestra ovalis, are sensitive to particle motion (i.e., a velocity detector—Fay et al. 1974). Could that structural organization, centering on the macula neglecta, somehow impart directional information about a distant sound source? Further speculation is unjustified until more data confirm the system as part of the auditory system of sharks.

CONCLUSIONS

There can no longer be any doubt that sound plays an important role in the lives of sharks. It is used by them to locate food sources and possibly even other objects, such as competitors and predators. Results from studies using acoustical playback techniques have shown that repetitively pulsed, synthesized or naturally produced sounds, possessing frequency bands below 800 to 1000 Hz, are attractive to many species from a variety of habitats. Probably such sounds will eventually be found to be attractive to most, if not all, sharks. As one lowers the spectral content, attractiveness increases until an optimum is reached at very low frequencies, i.e., 40 Hz or below. Attractiveness increases also as repetitive pulsing increases (at least to 20 pulses/s), with irregular pulses being more effective than regular pulse trains. Pure tones and continuous sounds are not attractive to sharks.

Sharks perform a wide variety of behavioral activities in the vicinity of sound projectors. The technique of acoustic attraction therefore provides an opportunity to observe repeatedly various patterns of movements at times other than those of fortuitous encounters or when the animals have become highly excited by food used to attract them.

Under specific circumstances sounds can also elicit rapid withdrawal by sharks. Although much work remains to be done before this relationship can be described fully, it appears that some factor of intensity is central. There is evidence indicating that absolute intensity of sound is not the

critical point; rather, it is the manner whereby a given intensity is reached, i.e., rapidly or slowly, relative to some unknown reference. This area of research seems highly promising for reasons of biological interpretation and because of its general implications with regard to present theories of withdrawal processes in other animal groups.

The numerous theoretical and practical difficulties that have faced scientists during recent years regarding the problem of directional hearing in fishes seem now to have been alleviated, at least in part. There is presently good evidence that at least some teleosts and sharks do possess directional hearing, and as research proceeds the number of species demonstrating this ability will surely increase. Interest appears now to be shifting to the morphological and physiological mechanisms that impart directionality to hearing in these animals. There are a number of hypotheses and very few facts. Testing these and other ideas will be an important area of research for the next few years. Such research, it is hoped, will include sharks and other elasmobranchs among it subjects.

ACKNOWLEDGMENTS

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TELEMETERING TECHNIQUES FOR THE STUDY OF

FREE-RANGING SHARKS

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INTRODUCTION

Recent advances in acoustic and radio telemetry have made these techniques increasingly useful to biologists conducting field studies of the behavior patterns and underlying sensory mechanisms of terrestrial and marine animals. In the case of free-ranging sharks, especially the larger active species, telemetering techniques are particularly needed because of the difficulty of studying these animals by direct underwater observation. Such difficulties stem from sharks being generally (1) shy of divers unless attracted by bait, (2) wide ranging, and (3) in a concealing environment, i.e., visibility a small fraction of home-range dimensions. In addition, many species are usually too deep for scuba observation, and most species are active primarily at night. There is also a significant danger to the unprotected observer in certain circumstances, e.g., agonistic attack by gray reef sharks (Johnson and Nelson 1973).

This review is intended primarily for biologists contemplating the use of telemetry in field studies of sharks or other large marine animals. Present capabilities and future possibilities are discussed, with example taken mainly from shark-telemetry studies in which the author has been involved. This paper is based partly on and includes some modified excerpts from the previous article "Ultrasonic Telemetry of Shark Behavior" (Nelson 1974).

The main emphasis is on *ultrasonic* telemetry, as only acoustic transmission is practical from fully submerged, free-ranging sharks in seawater. *Radio* telemetry is considered, however, for the special application of non-continuous trackings using timed-release radio floats. Such radio methods appear most useful for monitoring relatively long-term movements or migrations.

For a broad coverage of the techniques of telemetry in biological research, the reader is referred to the second edition of *Bio-medical Telemetry* (Mackay 1970). Although written prior to some of the current electronic technology (e.g., CMOS micropower digital integrated circuits), the book contains a wealth of information, ideas, and circuits and should be considered a primary reference work for those involved with biotelemetry. Mackay's emphasis is on radio methods, such as low-power transmission of physiological data from inside the body to outside, but both long-range field applications and ultrasonic methods are treated. The reader is also referred to the "Underwater Telemetry Newsletter"[1] for up-to-date, practical information on the telemetering techniques (mainly ultrasonic) used in tracking and monitoring aquatic animals. Each issue also contains news of biological studies and a bibliographic updating of publications in underwater biotelemetry. An annotated form of this bibliography is available elsewhere (Stasko 1975), as well as a more recent review of the subject (Stasko and Pincock 1977).

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[1]"Underwater Telemetry Newsletter" is presently edited by Charles C. Coutant, Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee 37830. It has been distributed to interested researchers approximately twice a year since 1971.

For several years, a development effort has been under way at California State University, Long Beach (CSULB), aimed at producing a system for telemetering several aspects of the behavior of free-ranging sharks at sea. In contrast to the simpler "pingers" used in most previous fish-tracking studies, this objective required a relatively powerful, complex transmitter incorporating several sensors and associated switching components. Standora (1972) described the multichannel ultrasonic transmitter developed at CSULB and its initial use in 1971-1972 for monitoring the day and night behavior patterns of the Pacific angel shark at Santa Catalina Island, Calif. His transmitters incorporated sensors for measuring depth, swimming speed, light, and temperature, and the multiplexed data were tape recorded for later manual decoding. Nine angel sharks were tagged underwater (via barbed dart) and afterwards tracked from a boat for 13 to 25 h. The sharks were nocturnal in activity, swam at depths of 27 to 100 m, and confined their movements to a home area of about 150 hectares (Standora and Nelson 1977).

Sciarrotta (1974) and Sciarrotta and Nelson (1977), using an improved version of the CSULB multichannel transmitter, studied the blue shark (Figure 1), a dangerous species abundant in offshore temperate waters. Units containing various combinations of depth, temperature, swimming-speed, and compass-heading sensors were externally applied to free-swimming sharks baited to the boat several kilometers off Santa Catalina Island. Telemetered data showed that the sharks were generally more active at night than during the day, with swim speed, rate of change of direction, and rate of change of depth all greater at night. The most interesting behavior discovered was a well-oriented shoreward migration to the island at dusk (Figure 2). The sharks typically stayed near the island for several hours, then gradually moved back out to sea. Deviation from straight-line swimming was greatest upon the early evening arrival at the island, and it was postulated that feeding may have occurred then.

A number of multi-day telemetry trackings were conducted at Rangiroa, French Polynesia during 1973–1975 as part of an overall study of the reef sharks of the area (Nelson and Johnson, in press). Using both depth-sensing and location-only transmitters, the units were concealed in bait and fed to uncaptured sharks in a manner resulting in completely atraumatic application. Home ranges and diel patterns of activity and movement were determined for gray reef sharks (Johnson and Nelson, in preparation) and reef whitetip sharks (Nelson and Johnson, in preparation).

ULTRASONIC TECHNIQUES

Telemetry from fish in the natural environment has traditionally involved *acoustic* (ultrasonic) transmission, as *radio* waves are greatly attenuated by water, especially high-conductivity seawater. Although radio techniques have recently been used for tracking free-ranging fish, e.g., salmon, trout, and bass (Winter et al. 1973, Monan et al. 1975), they are practical only in freshwater

Figure 1   Blue sharks (*Prionace glauca*) tagged with Mark III multichannel ultrasonic transmitters with drogue-type speed sensors. Center photo shows a large individual, about 2.5 m long, returning to the bait canister immediately after tag application. (Photos by T. Sciarrotta.)

at relatively shallow depths. One advantage of radio over ultrasound is that signal propagation is not adversely affected in areas of high bubble entrainment such as below waterfalls. Radio telemetry from animals in seawater is possible but only at very low frequencies and at very short ranges. Mackay (1970) discusses successful transmission from a dolphin in a pool 15.2 m (50 ft) in diameter and 2.1 m (7 ft) deep. In this case, of course, acoustic telemetry could not be used because of the hearing sensitivity of the animal in the ultrasonic region—a problem that is nonexistent with fish. For the continuous tracking of free-ranging sharks in the marine environment, the distances and depths are such that only acoustic transmission is possible.

Furthermore, for the relatively long ranges normally required in field studies, only *pulsed* transmission at relatively low duty cycles is practical for reasons of power conservation. The power-consuming transmitter output stage is thus on only for the duration of the pulse, a relatively small percentage of the time. With *continuous* transmission, the power-output stage is on 100% of the time, and the carrier frequency produced is usually modulated in amplitude or frequency by the information to be telemetered. Continuous-transmission telemetry is normally used at very short ranges, usually on captive animals, to monitor rapidly changing physiological variables such as the electrical activity of the heart (ECG waveform), respiratory
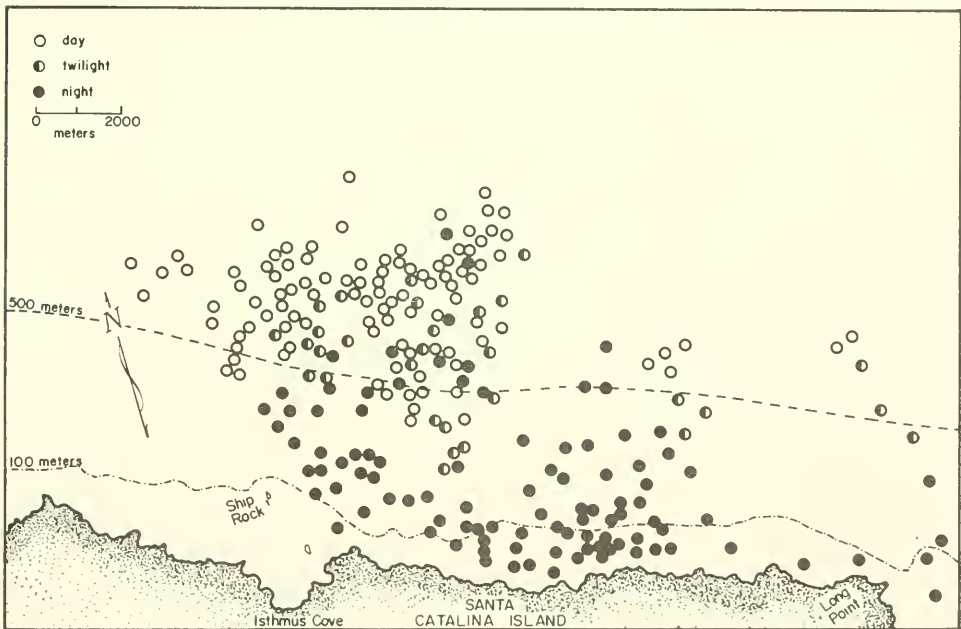
Figure 2a Positions of seven blue sharks tracked by T. Sciarrotta during the spring season of 1972. Note that day positions are well away from the island, while the majority of night positions are near shore. (From Sciarrotta and Nelson 1977.)
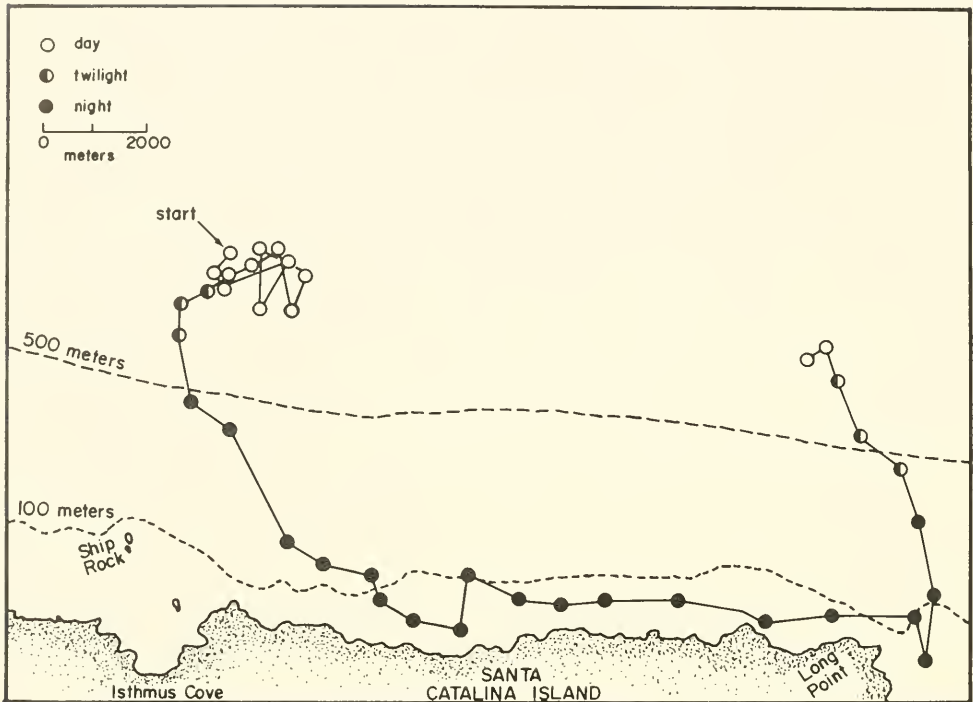
Figure 2b Individual track (approx. 0.5-h intervals) of one of the above sharks, starting at 1200 h, ending at 0600 h. (From Sciarrotta and Nelson 1977.)

movements, or pressure fluctuations in the gastrointestinal tract. Techniques of this type are treated by Mackay (1970) and by Kanwisher et al. (1974). An example of continuous-transmission FM telemetry from a shark is the study of Kotchabhakdi et al. (1973), who monitored electrical activity from the brains of free-swimming dogfish in a large pen.

Because this article addresses mainly the problems of *long-range* telemetry from free-ranging sharks in the natural environment, only techniques of pulsed transmission will be discussed further.

Types of Ultrasonic Transmitters

Figure 3 diagrams the signal parameters used in the following discussion of pulsed ultrasonic transmitters (USTs). Generally, when sensor data have been transmitted, they have been encoded as variations in pulse rate (pulse interval). Pulse-length variation is a poor way to encode data, as propagation of the sound through the water can time-stretch and distort the received pulse, especially if multipath problems are serious. Frequency variation has likewise not been used as a means of encoding sensor data from small, pulsed USTs, although it is used to distinguish between individual transmitters (each at a different frequency) or between channels in some two-channel trans-mitters.
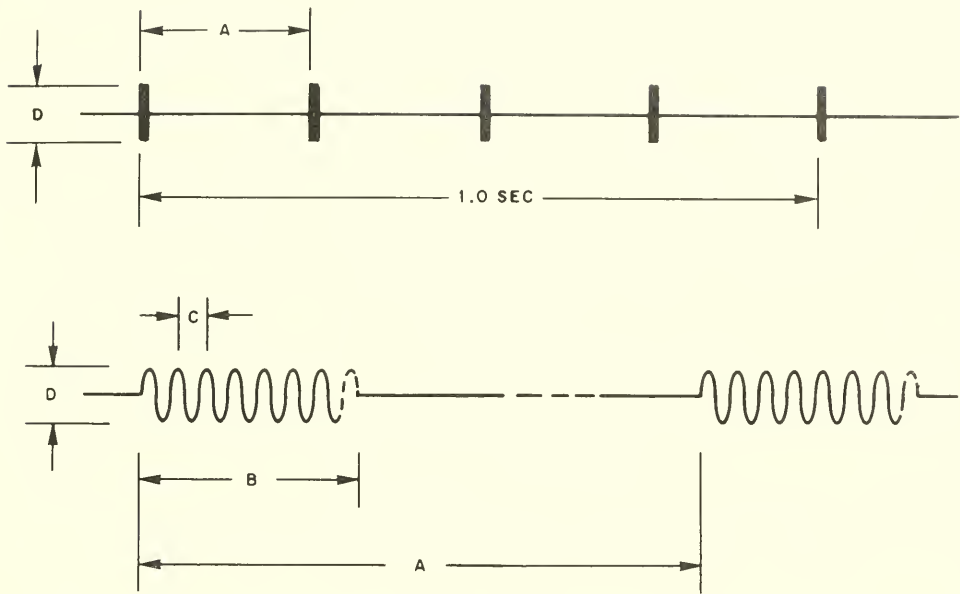
Figure 3 Basic signal parameters for pulsed ultrasonic transmitters. Oscillographic representation of signal pulses (tone bursts) of frequency, 40 kHz; pulse length, 10 ms; and pulse rate, 4/s.

A. Pulse interval (0.25 s). Pulse rate is the reciprocal of pulse interval, $1/0.25 = 4.0/s$ pulse rate.
B. Pulse length (pulse width), e.g., 10 ms.
C. Length of one cycle (0.025 ms). Frequency is the reciprocal of cycle length, $1/0.025 = 40$ kHz frequency.
D. Pulse amplitude (peak-to-peak), corresponding to sound pressure in the medium or to voltage in the hydrophone, receiver, or oscilloscope. Volts (root mean square) = volts (peak-to-peak)/2.83.

Location-Only (Pinger)—The simplest types of USTs are those without sensors—often referred to as "pingers"—which transmit pulses at a relatively fixed rate. Units of this type were used by Bass and Rascovich (1965), who were the first to show the feasibility of tracking large, fast-swimming fishes (tuna, sharks) from a moving vessel at sea. Since the general location of the tagged individual is the only information obtainable from a pinger, instabilities in the frequency or pulse rate are of no consequence unless they adversely affect signal reception or the ability to distinguish between units.

Simultaneous tracking of several recognizable individuals requires that each pinger be set at a different frequency and/or pulse rate; these should be reasonably stable as temperature, pressure, and battery voltage vary. Constant frequency is particularly important because drifting frequency can lead to confusion in distinguishing individual units. In addition, if the frequency drifts away from the expected, especially after a prolonged tracking intermission, the trackers may fail to regain the signal because they are not

listening at the right frequency. Furthermore, with directional receivers, a relatively slow 360° scan of the "underwater horizon" is required to regain a lost signal. If the frequency of the lost signal is uncertain, then the tracker must make a separate scan at each of many different frequencies—a prohibitively time-consuming process. This problem of missing the correct frequency is most critical with the narrowband receivers that are usually preferred in tracking operations because of their greater noise-rejection capability.

Serious consideration should therefore be given to the frequency stability of any UST, even one intended only for general location tracking. While some very simple, inexpensive pinger designs are available, they may suffer from frequency variations in response to variations in temperature or battery voltage. Extremely stable frequencies can now be obtained by quartz-crystal control, such as in the CSULB Mark V transmitter diagrammed in Figure 4.
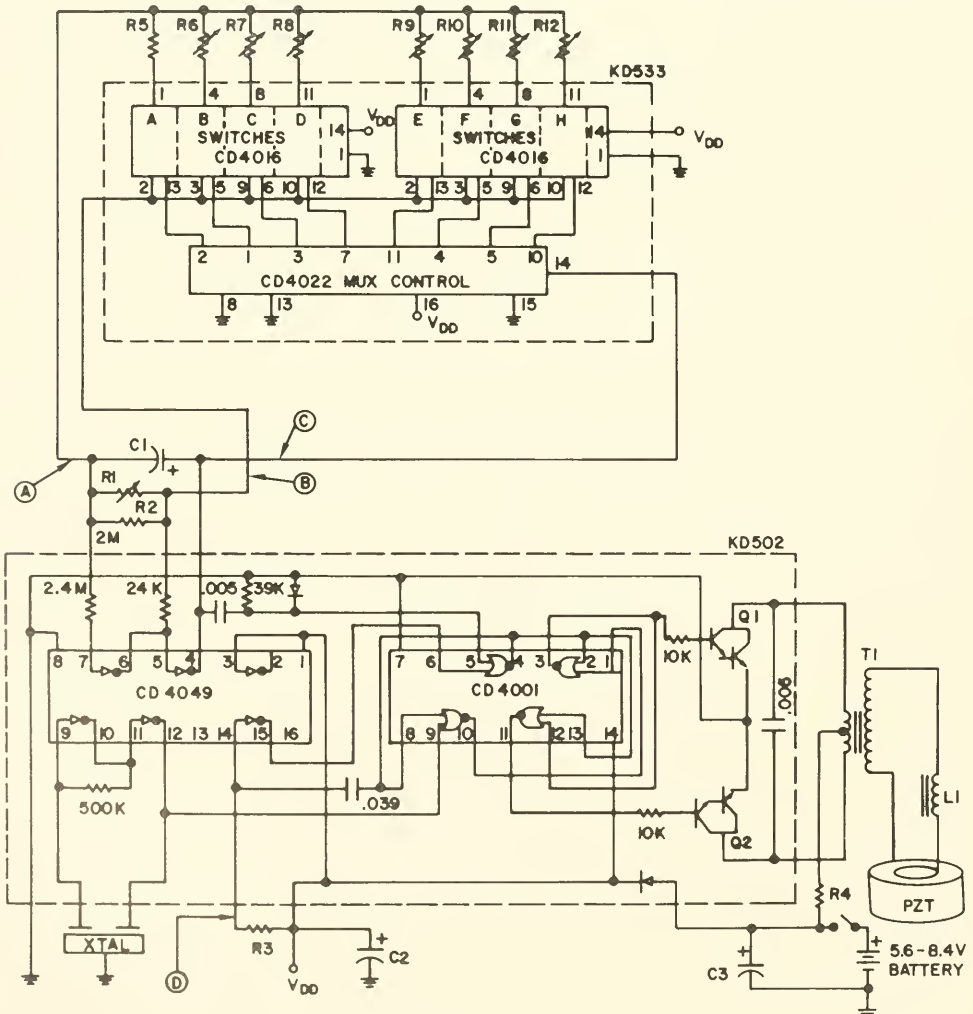
Single-Channel (One Sensor)—In recent years, biologists have been increasingly interested in including one or more sensors in the transmitter package, so as to obtain data parameters in addition to the animal's location. In many UST circuits, replacing one fixed resistor with a resistor that varies with the desired parameter converts a location-only unit into a one-channel data transmitter. A simple example is the incorporation of a thermistor that causes the transmitter to change pulse rate with temperature. When data are encoded as pulse rate, identification of individual USTs must be done by other means, usually frequency differences.

To telemeter sensor data accurately, the transmitter circuit itself must have high pulse-rate stability over the expected range of battery voltages and environmental variables. Thus, the degree of stability adequate for a simple pinger may be inadequate for a unit used to telemeter sensor data. For example, the Mark V shark transmitter (Figure 4) was specifically designed

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Figure 4   Circuit of the CSULB Mark V ultrasonic transmitters using CMOS digital integrated circuits, quartz-crystal frequency control, and hybrid thick-film construction. Basic transmitter components are in one hybrid (Keldron KD502), the multiplexer (mux) is in another hybrid (KD533), and some additional parts remain external for parameter adjustment, etc. For operation as a pinger (no sensor), omit KD533 and use a fixed resistor in place of R1 and R2. For single-channel operation, omit KD533 and use the sensor at R1. For multichannel operation (rapid-mux format), remove R1 and use the sensors and/or fixed resistors at R6–R12. For multichannel operation (slow-mux format), separate the multiplexer from the main transmitter at point C and, instead, drive the mux clock input (at point C) with a separate clock oscillator at the desired switching rate.

R1.	Pulse-rate control. The sensor in single-channel operation; unaffected by battery voltage (approx. 50–300 k; 150 k = 2.2/s, 300 k = 1.2/s).
R2.	Fail-safe resistor. If desired, to ensure continued operation if the sensor fails by opening circuit (approx. 1–2 m).
R3.	Pulse length control (approx. 560 k = 10 ms, 270 k = 5 ms; pulse lengths increase somewhat as voltage decreases).
R4.	Power-limiting resistor. If desired, to limit the current to the power amplifier to extend battery life (few ohms–few tens of ohms).
R5.	Reference-channel resistor for multichannel operation (chosen to be lower in resistance than any possible sensor value).
R6–R12.	The sensors for multichannel operation, i.e., the seven data channels.
C1.	Pulse-rate control (approx. 1.0 μF tantalum).
C2.	Logic power boost (approx. 120-μF tantalum).
C3.	Main power boost (approx. 360–510 μF tantalum).
XTAL.	Statek SX-1H (various frequencies near 40 kHz).
Q1, Q2.	Darlington power transistors (GE D40C1 or pairs of 2N2222's).
T1.	Primary, 80T CT #32; secondary 93T #34 (Ferroxcube 1408PL003B7).
L1.	92T #36 (Ferroxcube 1107CA2503B7).
PZT.	Thin-walled cylinder of PZT-4. OD 22.2 mm (0.875 in.); ID 19.8 mm (0.780 in.); height 12.7 mm (0.5 in.).

All capacitors in microfarads; all diodes, 1N914.

For external triggering of an output pulse (as in some timefix or transponding operations), apply a logic low at point D. The minimum pulse length will be the length of the externally applied low. Additional pulse length is obtained by upward adjustment of R3.

to be used with sensors (variable-resistor types) and thus has good pulse-rate stability.

Other examples of circuits usable as either pingers or single-channel USTs are given by Mackay (1970) and by various contributors to the "Underwater Telemetry Newsletter." Circuits for miniature depth-sensing transmitters using the strain-guage, bridge-type pressure sensors are given by Luke et al. (1973) and by Pincock and Luke (1975).

Multichannel—There are various methods by which data from several sensors can be simultaneously telemetered from a single transmitter. One way would be to encode Sensor 1 as pulse rate, Sensor 2 as pulse length, Sensor 3 as frequency, and so forth, but this method has not been used because of the problems associated with reception of pulse length and frequency. Another method would involve transmission of each sensor on a separate frequency channel, but this would involve the added complication of a multifrequency or FM/FM (frequency-multiplexed) transmitting and receiving system. To the author's knowledge, a system of this type has never been used on free-ranging marine animals. The preferred method appears to be time multiplexing, in which only one output frequency is used, but the information from the sensors is sequentially switched onto that one frequency channel.

An example of a multiplexed UST is the eight-channel transmitter developed at CSULB for monitoring the behavior of sharks at sea, which has been described in detail by Ferrel et al. (1974). An updated version of the circuit is shown in Figure 4. Up to seven sensors can be incorporated, the remaining channel being used as a reference for sequence identification. In the *rapid-multiplexing* format, each sensor controls pulse rate for just one pulse interval, then the next sensor is switched in. The resulting data (Figure 5) are in the form of a series of eight-interval data frames, each frame covering about 2–3 s. In the *slow-multiplexing* format, the channels are switched at a preselected clock rate, e.g., once each 10 s, allowing decoding of data by manual stopwatch timing. As in the previous example, a reference channel identifies the sequence of the other seven channels. This channel is set at a fixed pulse rate beyond the range of the data channels, e.g., reference interval, 150 ms; data intervals, 250–500 ms.

A different method of distinguishing between multiplexed channels was used in the two-channel temperature transmitter used by Carey and Lawson (1973) for comparing surface and deep-body temperatures in free-swimming tuna and sharks. This unit switched between sensors at the rate of once each minute, and channel identification was accomplished by slight frequency shifting (about 300 Hz) of the 21-kHz output frequency for one of the channels. Thus, without changing the receiver tuning, tracking personnel could determine the channel by the distinct shift in tone of the received 50-ms pulses.

Acoustic-Command Function (e.g., Transponding)—There are several advantages in having an acoustic-command capability that allows the tracker to control certain functions of a transmitter while it is being carried by a
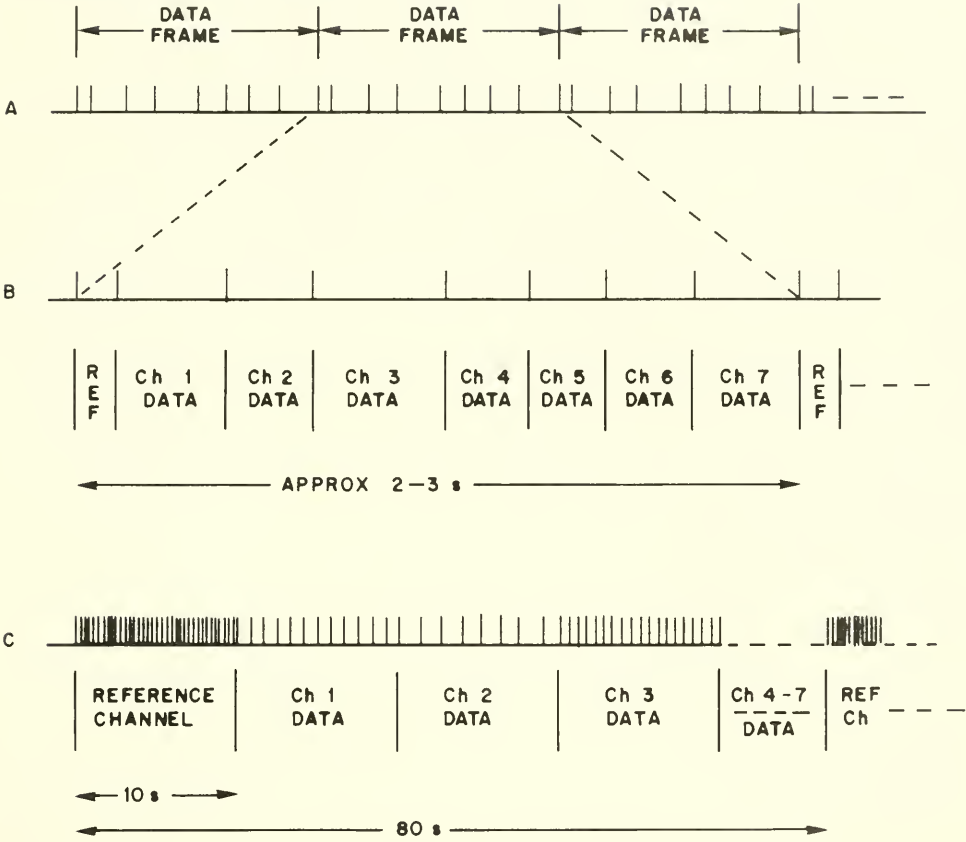
Figure 5 Data formats for the eight-channel CSULB ultrasonic transmitters: (A) Rapid multiplexing (one pulse interval per sensor), with three data frames shown. (B) Enlargement of one data frame from A, shows one reference channel and seven data channels. (C) Slow multiplexing (fixed time per sensor), in this case shown as 10 s per channel, thereby 80 s per full data sequence.

free-ranging shark. One such function is transponding, in which *interrogation* by the boat unit causes *reply* by the shark unit. The interrogate and reply frequencies are normally different to prevent the shark unit's responding to its own output. The primary advantage of transponding is that it permits accurate measurement of shark-to-boat distance, thus facilitating more precise determination of the shark's position. Receiving equipment in the boat provides this distance as a function of transponder reply time, based on the speed of sound in seawater (1535 m/s at 25°C, 35 parts per thousand salinity, and surface pressure).

A simple transponder for this purpose can be made by adding a small receiver/threshold detector circuit to any existing UST. Reception of an adequate interrogate signal triggers an additional normal transmitter pulse (the reply pulse). Recognition of this reply pulse and measurement of its

arrival time (re: interrogate signal departure time) permits the desired distance determination. A capability of this type is being developed for addition to the standard CSULB Mark V transmitters. In addition to the boat-based receiver/interrogate unit, a diver-held underwater unit will be used to allow easy interception of the shark for the purpose of making visual observations.

A transponding transmitter intended primarily for long-term use with home-ranging species of reef sharks is also under development at CSULB (Figure 6). The shark unit will be normally silent (listening mode) until it receives the proper interrogation signal. Then it will switch to the transmit-listening mode, remaining there for about 1 h past its last interrogation. This facilitates unbroken transmission without having repeatedly to interrogate the unit to keep it on. In this mode, the shark unit will respond to interrogation with its special reply pulse (frequency shifted) from which distance is obtained. If no interrogation is received for 1 h, the shark unit switches back to the listening mode, thus conserving power during the times the trackers are not present.

Battery conservation can be an important advantage of transponding as listening (receiving) circuitry can be made to draw relatively little power. For long-term studies where only intermittent checks on the telemetered subject are planned, useful transmitter lives of several months or more can be obtained while still retaining high acoustic power during on-times.

The degree of battery savings possible with transponding, however, depends on the normal power drain of the basic transmitter. As pointed out by Pincock and Luke (1975), for some small, low-power USTs, addition of transponding can actually increase the battery drain, even though the output
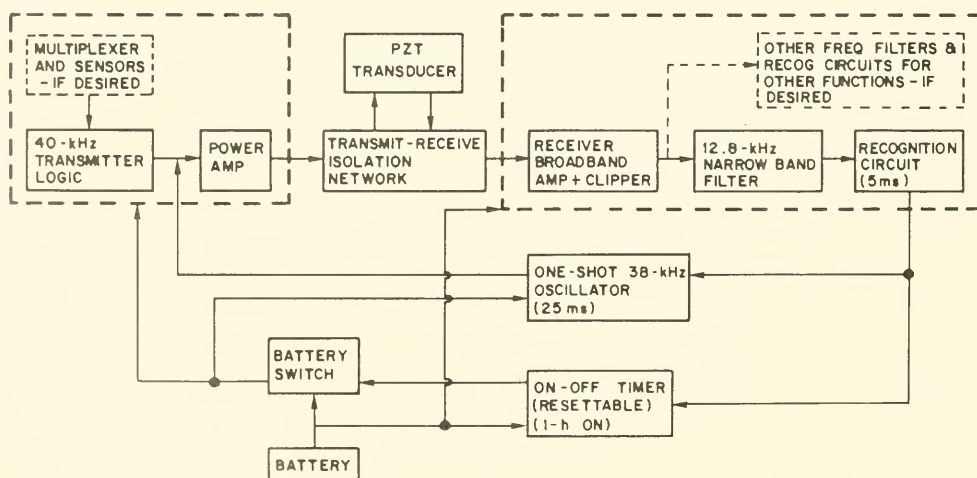
Figure 6   Functional diagram for a transponding transmitter. Interrogate frequency, 12.8 kHz; reply frequency, 38 kHz; data-transmit frequency, 40 kHz. In the listening mode, power is connected only to the receiver and timer sections.

stage is off most of the time. This is because the relatively low-drain receiver circuit (on 100% of the time) still has an average drain exceeding that of the power-output stage (which is on a small percent of the time, perhaps 1%). Thus, a net power savings can usually be achieved only for transmitters which are of relatively high average power such as the CSULB shark units. A further means of lowering power drain, as suggested by M. Yerbury (personal communication), would be to impose an on-off duty cycle on the receiver itself, this being controlled by an even lower power clock circuit. For example, the transponding receiver might be turned on for only 10 ms every 100 ms and thus would still be able to respond to any interrogate pulse more than 100 ms in length.

Besides transponding, other command functions of potential use are (1) interrogation of memory bank (see next section), (2) release of transmitter from shark, and (3) delivery of stimulus to shark for experimental purposes. Recovery of stomach-implanted transmitters might be possible by a command-regurgitate mechanism which releases an emetic. If several different command functions are included in one transmitter, the interrogate signals must be frequency coded for proper recognition.

Clock-Timed Function (e.g., Timefix)—Some of the advantages of transponding can be achieved without the need for interrogation by using timed functions within the transmitter. One example is battery conservation. If it is not intended to track a shark continuously but only at certain predetermined hours of day or night, the transmitter can use a micropower clock circuit to turn the transmit section on and off at the desired times. For instance, in a long-term study of home-range stability in a reef shark, it might be necessary to check the shark's position only once a day. The unit could transmit perhaps only 1 h each day, such as between 0800 and 0900 each morning, thereby increasing battery life by a factor of nearly 24.

In regard to distance determination, the "timefix" system developed by the fish-telemetry group at Trondheim, Norway, is of considerable interest (Holand et al., 1974). In timefix USTs, the timing of output pulses is very accurately controlled by a 1-Hz quartz-crystal circuit similar to that in a digital wristwatch. A similar 1-Hz oscillator at the receiver is initially set in exact synchronization with the unit to be applied to the fish. Therefore, any delay in reception of the fish pulse is due to the time needed for that pulse to travel through the water and is thus a measure of distance to the fish. The usefulness of this kind of system, of course, depends greatly on the accuracy (or predictability) of the two oscillators. The longer the time since the initial synchronization, the more uncertain the range information obtained, especially if the temperatures of the two units differ by a substantial but unknown amount. This temperature differential will probably be the main source of error in a timefix system for fish tracking, therefore the temperature coefficients of the crystals become important criteria for their selection.

Assuming a frequency difference between the two clock oscillators of 10 ppm (equivalent to a drift of 1 s/day), a range-accuracy drift of about

1 m/min will occur,[2] therefore the range uncertainty after 1 h will be about 60 m (less any correction that might be applied). This amount may seem excessive for close-in positioning but would allow very useful estimations near maximum transmitter ranges of several kilometers. Furthermore, it would often be possible to resynchronize the system periodically, at least roughly, by other estimates based on visual contacts or by passing over the animal and noting the minimum time delay. Although the accuracy of *absolute* range estimates decline with time, the accuracy of short-term *changes* in range is not affected. Thus, small relative movements toward or away from the tracking boat can be determined, provided the short-term stability of the clock remains satisfactory.

A timefix circuit for the CSULB Mark V single-channel transmitter is shown in Figure 7. At the receiver, the reference clock oscillator is adjustable in phase, permitting easy initial synchronization and subsequent resynchronization of the system. The pulse-delay times are output on either an LED display or on a miniature storage oscilloscope.

In its simplest form, a timefix system does not allow pulse-interval coding of sensor data. However, there are ways of multiplexing such sensor data along with the time-synchronized clock pulses. One way is to switch alternately between the sensor and the timefix oscillator at some reasonably slow rate, e.g., once every 10 s, thereby permitting manual stopwatch decoding of the sensor data. Another, perhaps better, way would be to follow each timefix pulse with a sensor pulse using the interval between to encode sensor data, as described in Figure 7. In this case the pulse intervals must be measured automatically, or with an oscilloscope.

A clock-controlled transmitter having both timefix capability and programmable on-off duty cycles is currently under development at CSULB. The crystal-clock module provides both the 1-Hz timefix pulse to the transmitter circuit and an accurate time base to the 24-h on-off timer. The timer resets at exactly 24 h and can be set for various on-off schedules, e.g., 1 h on and 1 h off; 1 h on and 3 h off; 1 h on and 7 h off; 1 h on and 23 h off; etc. In the off mode, power is consumed only by the very-low-drain clock and timer circuits.

In comparison to a transponding system, the primary advantage of a timefix system lies in its simpler circuitry and operation, for both shark and boat units. Its 1-Hz oscillator also facilitates the addition of other clock-time functions. In other respects, transponding holds the advantage, providing maximum range accuracy throughout the tracking and permitting interrogation (or other commands) at any time during the tracking.

Clock-timed functions may be used for a variety of other purposes aboard the transmitter, such as scheduled "dumping" of stored sensor data or detaching the unit from the shark at a precise time. A further potential benefit of a clock-timed transmitter is that its extremely periodic pulse rate would lend itself to a receiver design using the principle of signal averaging, which

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[2]Under good conditions, much better accuracies are obtainable.
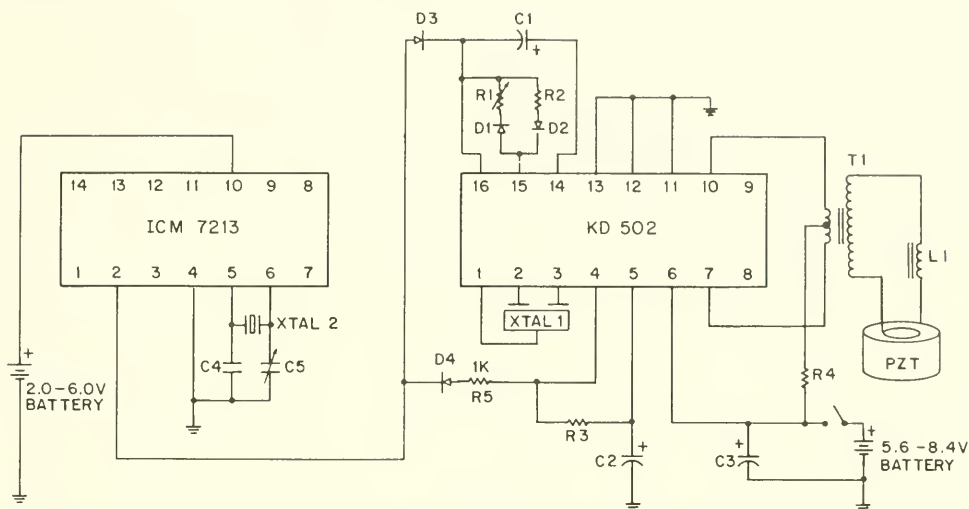
Figure 7 A sensor-compatible timefix version of the CSULB Mark V ultrasonic transmitter (see Figure 4 for more circuit details). Sensor data encoded as the interval between the *timefix* pulse (one/sec) and a *sensor* pulse following it by 200 to 400 msec (thereby distinguishable from it). The ICM7213 (Intersil, Inc.) is a CMOS precision clock oscillator/divider with a 1-Hz output. It's separate battery minimizes power drain (100 μA at 3.0V); if this not needed, the entire transmitter can operate from a single suitable supply, e.g., 5.6V.

In the circuit shown, R1 is the sensor (range chosen to give 200-400 msec interval), and R2 is much higher, e.g., 2M. In this mode, the minimum length of the timefix pulse is determined by the length of the clock output pulse (7.8 msec). Both timefix and sensor pulse lengths are increased by upward adjustment of R3, but the timefix pulse will be longer by about 7.8 msec.

To operate as a simple timefix unit (without sensor), leave pin 15 open and eliminate R1, R2, D1, D2, and D3 and its connection. Pulse length is controllable above 7.8 msec by R3.

C4, C5. Clock-timing capacitors (approx. 10–30 pf each)
XTAL 2. Clock crystal (A-T quartz, 4.194304 MHz)
D1, D2, D3, D4. 1N914

would effectively increase the signal-to-noise ratio, thereby improving reception under certain conditions.

Data Storage—The capacity to store sensor data would be a very valuable addition to the transmitter package for many studies, particularly long-term ones. A small memory circuit permitting remote interrogation would hold a very useful amount of data but not nearly the quantity that the transmitter could produce in 24 h. Thus, while full detail (sensor readouts several times a second) can be obtained only by continuous recording, this amount of detail may not be necessary for many research objectives.

If, for instance, the researcher is interested in a shark's general day-night activity pattern, it may suffice to know its mean swimming speed for each of the 24 h. Even more could be learned if an upper and lower range value

accompanied each hourly mean. Circuitry capable of processing a sensor's output and accumulating this much data (approximately 1000 binary bits) is presently planned for the shark transmitters. Upon receipt of the proper interrogate code from the boat, the shark unit would send back, in binary code, its previous 24 h of stored data via a nondestructive readout. The unit would then begin accumulating data as it arrives, discarding only that data older than 24 h. Thus, the shark unit need be interrogated only once each day, alleviating tracking fatigue and freeing the researchers for other work.

The above telemetry system is for remote, periodic interrogation of the data-storage module while the unit is still on the shark. An alternative (nontelemetric) method would be direct readout of the stored data after recovering the unit at the end of a tracking. For very high capacity storage, a small magnetic tape recorder may be preferable if space is available. The subminiature, micropower, digital-incremental tape recorder developed by Goodman et al. (1973) for animal-borne instrument packages measures about 100 cm^3, weighs about 100 g, and stores 20 million data bits. Many other data-recording methods exist (mechanical, electronic, photographic, and so forth), some of which are discussed by Mackay (1970).

Transmitter Construction

One problem facing the prospective user of telemetering techniques is a source of suitable transmitters. In the past, most research teams built their own units to meet their particular requirements. Nowadays, several companies offer basic USTs for sale, some with the capability of sensing temperature or pressure (not both in one unit). Information on most commercially available USTs has appeared at one time or another in the "Underwater Telemetry Newsletter." For many studies, such off-the-shelf units are perfectly adequate and probably most economical. Other research purposes, however, require specialized transmitters that are not available commercially and are therefore usually designed and built by the researchers themselves or on contract.

Methods of Circuit Construction—During the last few years, the electronics industry has undergone a "quiet revolution" in the development and proliferation of integrated circuits (ICs), a large variety of which are now available at low cost in various small multipin packages or as tiny chips. Of particular usefulness in telemetry are the CMOS (complementary metal-oxide semiconductor) digital types which have low-microwatt quiescent power requirements and operate over a wide range of unregulated voltages (< 3–15V). Using ICs, the designer can now incorporate a wider variety of functions (e.g., gating, counting, crystal-controlled timekeeping, multiplexing, data processing, and storage) into small, low-power circuit packages than would have been practical using earlier technologies. However, the primary benefit to biotelemetry of the new IC technology is to permit the addition of new, more complex functions to transmitters, not to achieve power savings in the transmitter's power-output stage.

These are several more or less standard methods for assembling modern solid-state circuits. These are listed in order of increasing miniaturization.

Discrete wiring—This is the "standard" method of circuit construction, in which the individual components are soldered together. Usually the neatest results are those utilizing a printed circuit (PC) board, although the most compact arrangements are sometimes those wired without such a board (Figure 8). Most USTs in the past have been built this way, and for very simple circuits there may be no significant size advantage in using a more sophisticated method.

Figure 8   Comparison of construction methods for CSULB Mark V transmitter circuits (single channel). Left: Discrete wiring of the compact boardless type. Right: Thick-film hybridization. The multipin hybrid contains most of the circuit components. The remaining external components include the frequency crystal (TO-5 can at top), capacitors, resistors, and a multipin connector.

Thick-film hybridization—This type of miniaturized circuit construction allows a considerable size reduction over discrete wiring. The conductor pattern is produced on a ceramic substrate by a "silk-screen" method, the smallest conductor lines and spaces being about 0.25 mm wide. Miniature components such as chip capacitors, integrated-circuit chips, other semiconductor dice, and external-connector pins are applied to the substrate. Additional connections are made by hand under a binocular microscope using 0.025-mm wire, usually attached using an ultrasonic or thermocompression wire-bonding machine. Resistors can be added as drops of resistive ink and later trimmed to desired values using an abrasive airbrush. The finished circuit is tested, then coated with a protective epoxy or otherwise mounted.

Since there is usually an initial setup fee, thick-film construction is generally not more economical than discrete wiring in quantities of fewer than

about 100 units. This, however, varies greatly with the companies involved. Examples of hybrid thick-film circuits are those produced for the CSULB shark transmitters by Keldron, Inc., Costa Mesa, Calif. (Figures 8 and 9), and those described by Pincock and Luke (1975) for a smaller, pressure-sensing transmitter.

Thin-film hybridization—This is a more sophisticated type of circuit miniaturization derived primarily from monolithic technology. It allows more compact packaging than thick-film hybridization but is somewhat more costly in any quantity and much more costly in quantities of fewer than about 1000. The smallest conductor lines and spaces are about 0.025 mm wide. Conductor and resistor patterns are produced by photo-etched masks, using deposition techniques such as evaporation and sputtering. Larger components are attached as in thick-film hybridization.
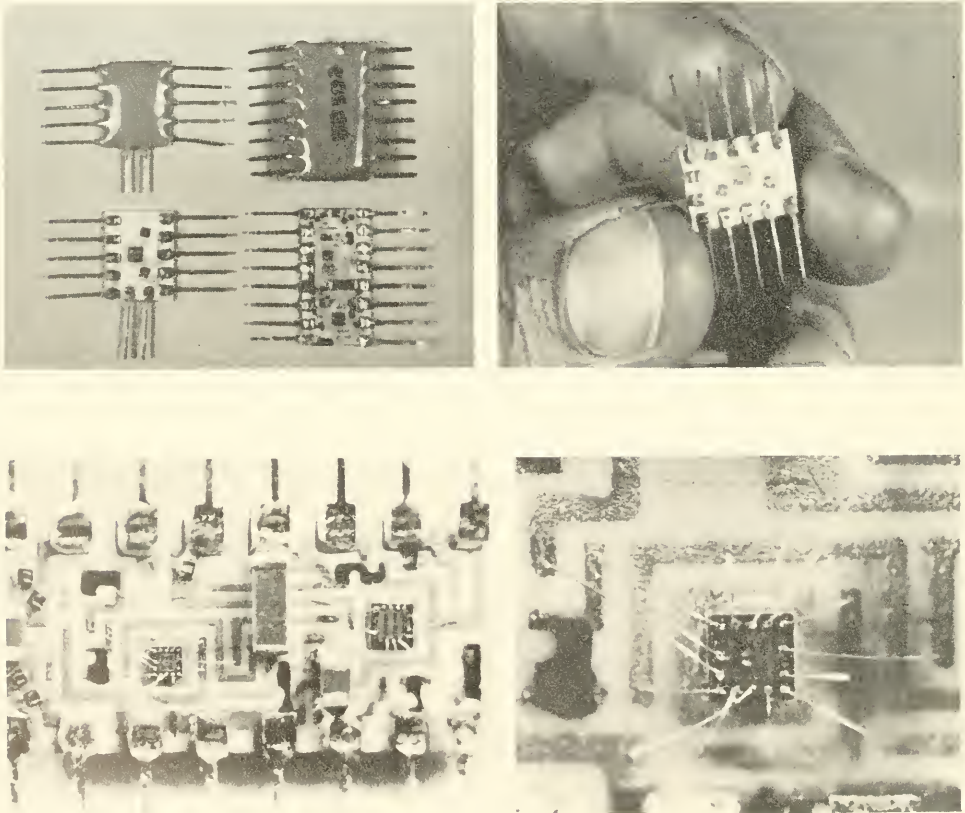
Figure 9   Examples of thick-film hybrid circuits. Top: Potted and unpotted hybrids for the Mark V basic transmitter (KD502, 16-pin) and the eight-channel multiplexer (KD533, 12-pin). Bottom: Closeup of details, including wire bonding of the CMOS integrated-circuit chips to the substrate conductors. Hybrids produced by Keldron, Inc., 1281 E. Logan Ave., Costa Mesa, Calif. 92626.

Monolithic integration—The maximum in microminiaturization is achieved by full integrated-circuit (IC) construction. The multilayered circuit is produced on a silicon wafer, with conductor lines and spaces down to about 0.0025-mm widths. All components are laid down by photomasking and diffusion deposition of the desired circuit materials. The resulting IC chips, although minute, cannot be used alone; they must be either incorporated in hybrid circuits or mounted in other relatively large packages to facilitate making standard connections to them.

The production of an entire special-purpose circuit (such as a multichannel UST) on a single IC chip would be economical only for quantities of more than about 20 000 to 50 000 units and would be prohibitively costly in the small quantities normally used for fish-tracking experiments.

Overall Size, Density—When considering how much to miniaturize a transmitter circuit, it should be kept in mind that there are usually certain components that cannot be miniaturized, e.g., battery, large capacitors, transducer, and transformer (Figure 8). Total transmitter size can therefore be reduced only so much by techniques such as hybridization and, for some simple USTs, these may not yield a significant overall size reduction. Thus, for the purpose of size reduction alone, hybridization may be worthwhile only for rather complex circuits. However, another benefit of hybridizing circuits is in the labor saved during final transmitter assembly. Having the hybrids produced by an outside company (as is usually done) makes for a simpler final wiring job. This is especially helpful to the researchers if they are doing this job themselves.

Another important consideration is the density (specific gravity) of the final transmitter package. Small transmitter size is obviously desirable, but the unit should also be close to neutral density, so as to neither weigh down nor lift up the animal. Many USTs turn out to be considerably heavier than water, especially those using relatively large mercury batteries. In such cases, it is questionable whether further size reduction (allowed by smaller circuits) is good or bad if it means an increase in submerged weight. It may be that a small, heavier-than-water unit may upset a fish's behavior more than a unit of the same mass that is somewhat larger, but closer to neutral density.

Packaging—There are several ways to house the transmitter components, the choice depending on factors such as cost, operating depth, and expendability. If the unit is to be recovered for repeated use, it should have a convenient means of entry for changing batteries or adjusting signal parameters. Also, since the more complex units are more likely to require troubleshooting, readjusting, etc., it is important that such units allow easy access to their various sections.

Potting—Transmitters and their batteries are often cast (potted) into one solid block of material such as wax, urethane, epoxy, or dental acrylic. While this method is very simple and yields minimum package size (but not

necessarily minimum weight), it has the disadvantage of making further circuit adjustment difficult or impossible. Potting, therefore, seems most applicable for expendable USTs with relatively simple, reliable circuits. Many commercially available USTs are of this type.

    Oil filling—The transmitter components can be housed in a relatively thin-walled plastic tube with rubber end caps and a filling of nonconductive oil, e.g., dehydrated castor oil. The compliant end caps allow pressure equalization between outside and inside, thereby permitting internal placement of the pressure sensor. The remaining components must also be able to withstand pressure. The main advantage of this method is simplicity; there is no need for a pressure-sealed housing. The primary disadvantages are greater
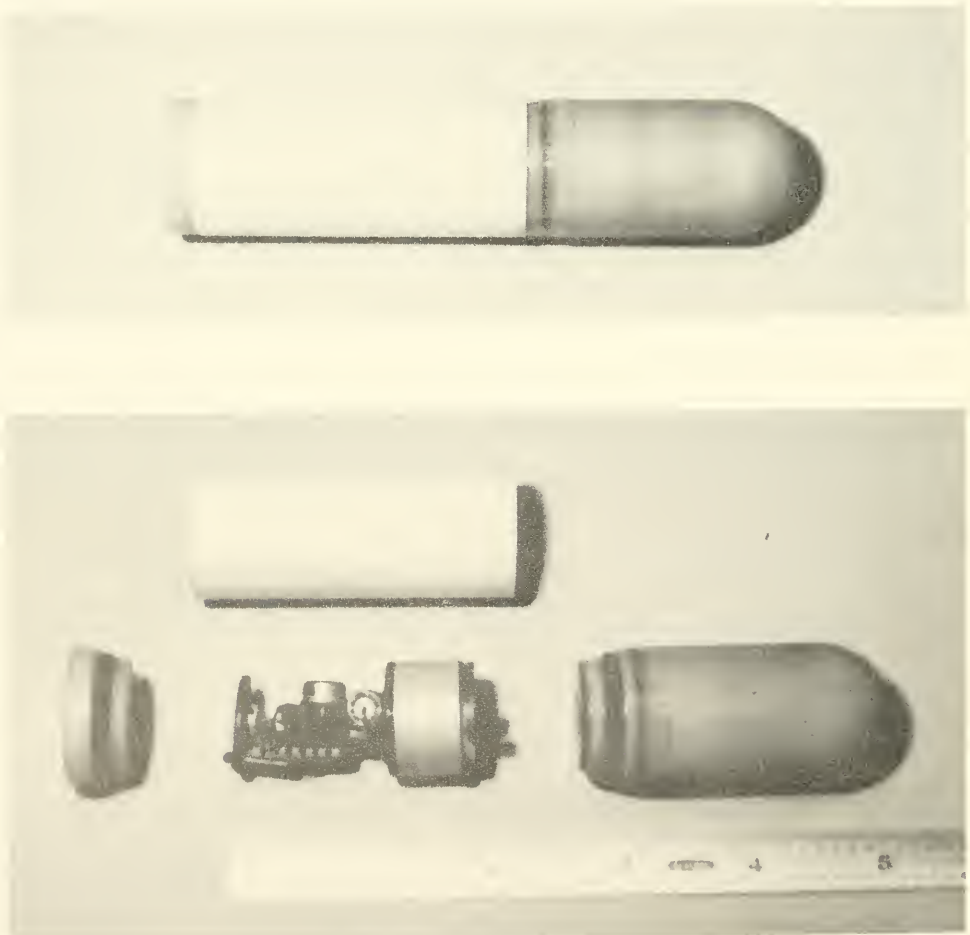
Figure 10 Mark V hybridized transmitter (no sensors) packaged in an air-filled, O-ring sealed housing. Right: Sealed battery module. Left: Plain end cap (in place of sensor module). Center: Circuit module plugged into transformer/PST assembly (before the latter potted into central tube).

weight and the extreme messiness when disassembling the unit for adjust-
ment or battery replacement. An example of this type of packaging is the
Mark III shark transmitter described by Ferrel et al. (1974).

   Air filling—An example of this type is the current Mark V shark
transmitter shown in Figures 10 and 11. A relatively thick-walled, pressure-
resistant tube is terminated on both ends by either glued or O-ring sealed
caps. Various material may be used; the author's preference is for plastic
because of its light weight, ease of machining, and incorrodibility. Ordinary
PVC pipe (sp gr = 1.4) is convenient, but ABS is better, being stiffer and
lighter (sp gr = 1.1). Acrylic or polycarbonate tubing (sp gr = 1.2) may be
used if transparency is desired. In air-filled housings, the acoustic transducer
element must be well coupled to the tube, such as by cementing it in with
epoxy as shown in Figure 11.

   The main advantage of an air-filled housing is ease of assembly and dis-
assembly. To facilitate easy removal of circuit sections, multipin plugs may
be used, as in the Mark V unit. Disadvantages include an operating depth
limit and a slight overall size increase because of the wall thickness of the
tube and end caps. However, submerged weight will generally be less than for
an equivalent oil-filled or potted unit. The probability of O-ring leakage is
very low, provided that the sealing surfaces are reasonably smooth and clean.

   If relatively few disassemblies are anticipated, it may be most convenient
to use simple glue-bonded butt joints. Flat joints (without internal shoulders)
are strong enough and can be cleanly separated with lathe and parting tool
with relatively little loss of material. Since glue-bonded end caps take less
tube length than O-ring sealed caps, an overall transmitter length reduction
can result, even if some extra material is left to allow for several possible
lathe partings.

   Sensors

If information other than the animal's location is desired, one or more
sensors must be included in the transmitter package. Many different param-
eters are measurable with the available technology. The following parameters
are those for which sensors have been built or are planned for the CSULB
shark transmitters. These sensors are of the variable-resistance type, having at
least a 2× resistance change in the general range of 50–300 kΩ. Some sensors
used in past shark transmitters are diagramed in Figure 12 and discussed by
Ferrel et al (1974).

   Temperature—Temperature can be directly sensed with a thermistor,
a device that changes resistance with temperature. In ordinary thermistors, as
temperature increases, resistance decreases, a typical coefficient being
4.6%/°C for a unit of 100 kΩ at 25°C.

   Light—Light also can be sensed directly by a single component, a
cadmium-sulfide photoresistive cell. However, the range of ambient-light in-
tensities found in the natural environment from day to night is much too
great to be covered by a single photocell. Instead, several cells of different
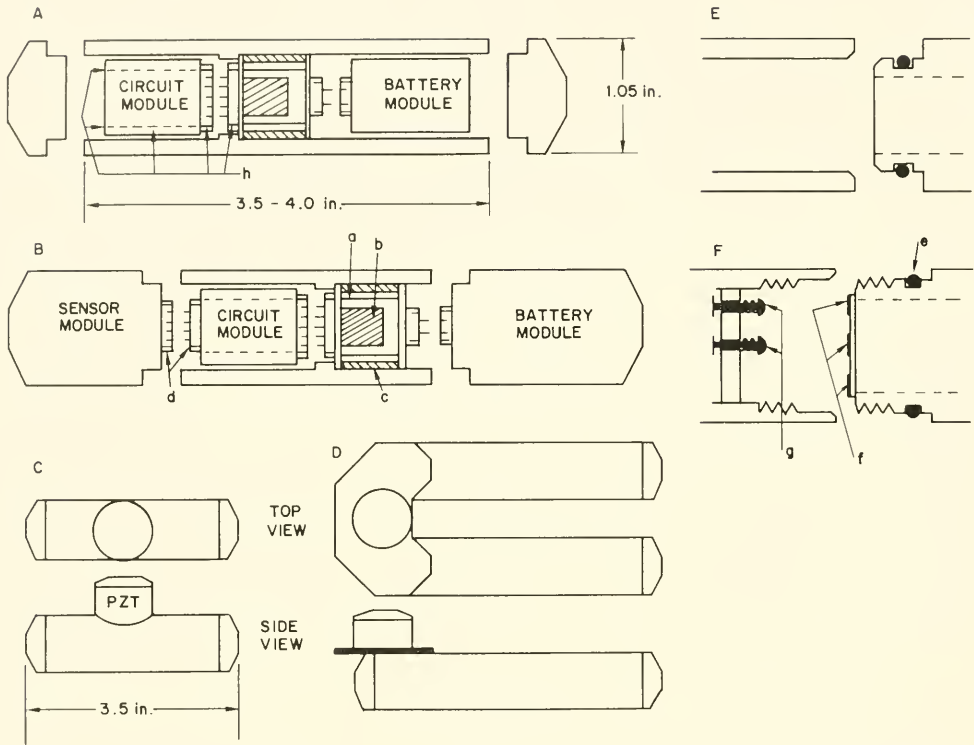
Figure 11   Various packaging arrangements for tubular, air-filled, O-ring sealed ultrasonic transmitters: (A) Single tube with plain end caps, removable circuit and battery. (B) Single tube with separate modules for battery and sensors, allowing use of various types of sensors and/or batteries with the basic transmitter module. (C) Single tube with PZT transducer mounted vertically for more omnidirectional radiation in the horizontal plane. (D) Twin-tube configuration for mounting on dorsal fin of shark (or to avoid a single tube of excessive length). (E) Sealing detail (simple push-in connection, held in by hydrostatic pressure and/or by tightness of the press-fit). (F) Sealing detail (two-turn threaded system, showing type of electrical contacts usable with a rotating connection). Diagrams not to scale.

   a. PZT acoustic transducer
   b. Transformer (in ferrite-cup core)
   c. Epoxy potting (between PZT and inside of tube)
   d. Miniature pin connectors (male and female)
   e. Neoprene O-ring
   f. Printed-circuit board with copper contact areas (outer ring, center disk)
   g. Spring-loaded electrical contacts
   h. Route of two long registration pins used as tools to align connectors when inserting
       circuit module.
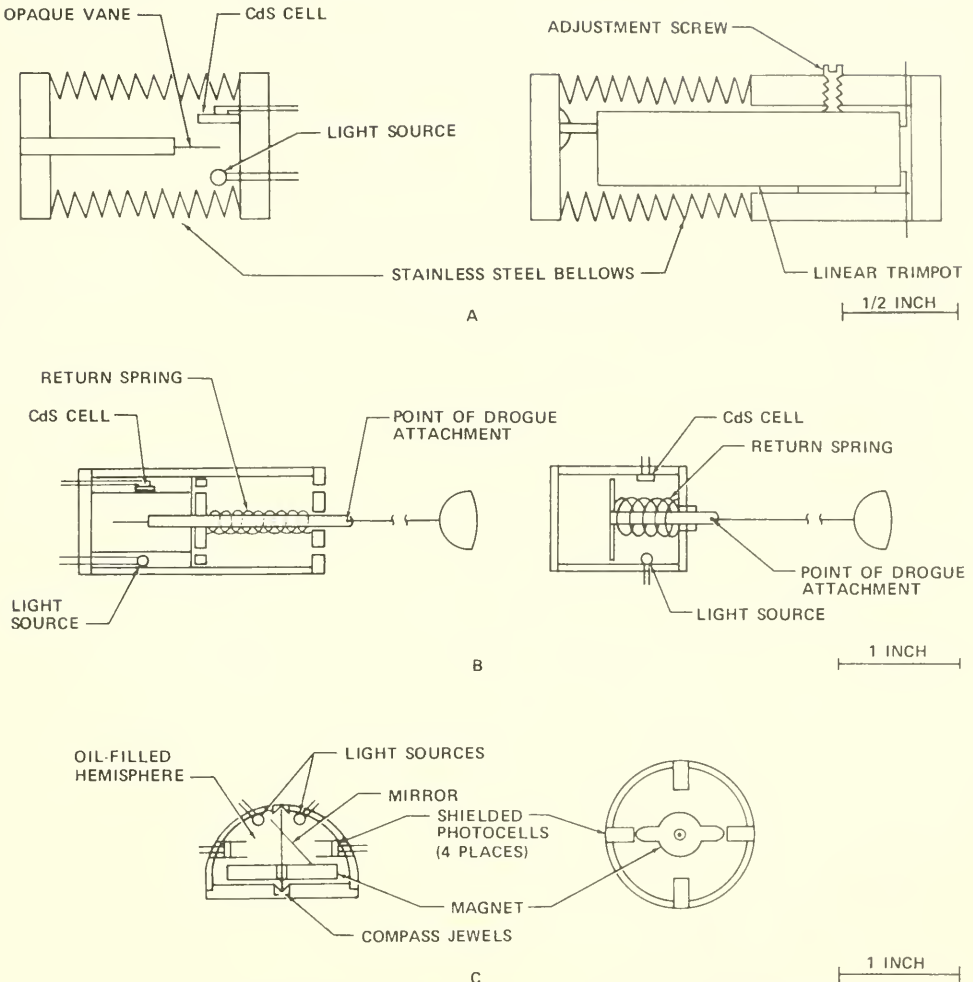
Figure 12 Some representative sensor types used in past CSULB shark transmitters: (A) Depth (pressure). (B) Swim speed (water flow). (C) Compass heading (azimuth). All act as variable resistors. The light/photocell types use low-power LEDs (light-emitting diodes) powered by zener-regulated voltage from the battery. The compass sensor requires four transmitter channels (one for each photocell).

sensitivities may be used on separate channels, or a network of photocells and resistors may be used on a single channel.

Depth—Depth cannot adequately be sensed directly by a single resistive component. In the shark units, a displacement proportional to depth is achieved by the compression of a sealed, air-filled metal bellows. This is converted into a resistance change by a linear trimpot or by moving an opaque vane between a light source (LED, betalight) and a photocell. In the present version, the bellows are partially filled with oil that limits compression and provides absolute protection against overpressure. Some bellows sensors are

two-stage units, having one thin-walled bellows for the shallow depths (0–20 m) and one thick-walled bellows for the greater depths (0–200 m). These may be operated on separate transmitter channels for maximum resolution or connected in series for one-channel operation (Figure 13).

Swimming Speed—Swimming speed is sensed by measuring the rate of water flow past the externally attached transmitter. Water force against a wand or drogue is converted to resistance change by a vane-photocell arrangement (Figures 12 and 14). One low-friction version operates by magnetic coupling of the external wand to an internal vane on a jewel-mounted pin. The wand-type sensor is mounted forward on the transmitter housing so that the wand encounters relatively smooth, turbulence-free water flow.

An indirect way to measure swimming speed is to relate it to tail-beat frequency. This can be measured by sensing lateral accelerations, such as in the tail-beat sensor described by Holland et al. (1974).

Swimming Direction—The instantaneous compass direction (azimuth) of the fish's axis at any moment can be sensed by a miniature magnetic compass sensor, one version of which is shown in Figure 12. A more recent version (Figure 15) uses a circular transparency gradient attached to the compass magnet and gives unambiguous azimuth readings by comparison of the resistances of two photocells mounted 90° apart, each

END VIEWS
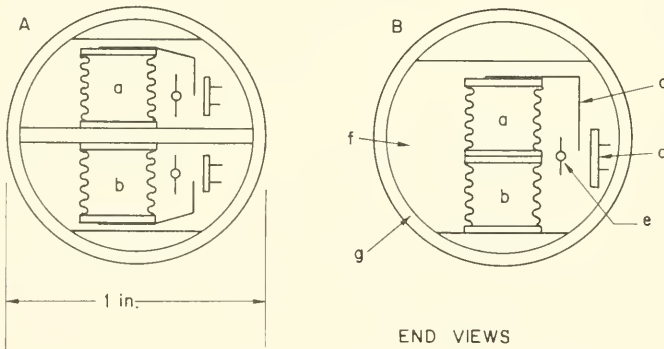
Figure 13 Depth sensors incorporating two partially oil-filled metal bellows. Designed to be impervious to over-pressure and to have relatively greater resolution at shallower than at deeper depths: (A) Twin-sensor unit for use on two separate transmitter channels. (B) Single-sensor unit for one transmitter channel.

    a. Thin-walled bellows (e.g., 90% compressed at 15 m)
    b. Thick-walled bellows (e.g., 90% compressed at 150 m)
    c. Opaque vane
    d. Photoresistive cell
    e. Light source (LED, betalight)
    f. Oil-filled chamber at ambient pressure (capped with opaque, compliant diaphragm)
    g. Thin-walled tube (not pressure resistant).
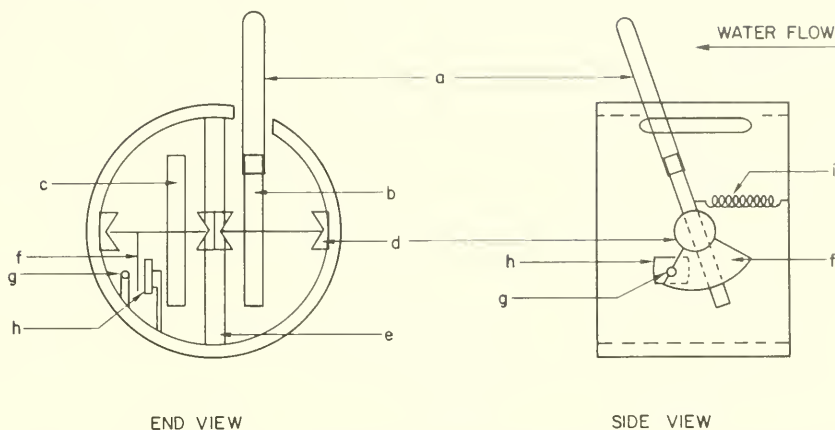
Figure 14   Wand-type swimming-speed sensor, magnetically coupled. Motion of the external magnet is duplicated by the internal magnet. System works with very low friction.

    a. Wand (senses water flow)
    b. External magnet (in water chamber)
    c. Internal magnet (in oil chamber)
    d. Compass jewel bearing
    e. Opaque partition (plastic)
    f. Vane (either opaque or of a transparency gradient)
    g. Light source (LED, betalight)
    h. Photoresistive cell (CdS)
    i. Spring.
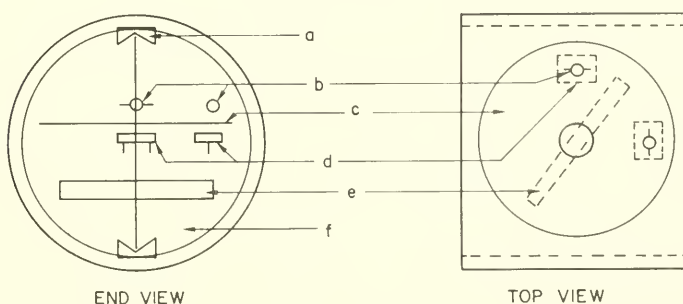
Figure 15   Compass sensor for telemetry of swimming direction. Comparison of the two photocell readings (two transmitter channels) allows unambiguous determination of azimuth. Employs a circular transparency gradient with no abrupt changes (sectors of maximum and minimum density are 180° apart).

    a. Compass jewel bearing
    b. Light sources (LEDs, betalights)
    c. Transparency gradient
    d. Photoresistive cells
    e. Compass magnet
    f. Oil-filled chamber.

connected to a separate channel. If ungimballed, the transmitter must be mounted level to ensure proper operation of the compass.

A wholly electronic compass sensor with no moving parts would seem preferable to the previously mentioned units but apparently presents problems in terms of cost, complexity, and power drain. The potential use of fluxgate magnetometers or "thin-film" magnetic detectors is mentioned by Mackay (1970).

Inclination—The tilt (pitch) of an animal's axis can be telemetered by a fluid-damped sensor in which a weighted pendulum moves a vane between a light source and a photocell.

Acceleration—Whole-body accelerations can be sensed by a pendulum-vane or spring-weight device with the correct kind of damping. Some accelerations may be very brief, e.g., a shark's final rush at prey, which may last only 1 s. In such a case, if a multichannel transmitter is being used, the multiplexer may not be on the appropriate sensor at the instant the event occurs. Thus, the sensor should have sufficient "persistence" to retain an indication of the event for at least one full data frame (approx. 2–3 s). This could be done hydraulically, with the accelerometer mass damped by a fluid dashpot (on the return stroke only).

The possibility of misinterpreting a brief acceleration as a brief change in tilt and vice versa also must be guarded against. Close examination of the data from both sensors should resolve any such ambiguity.

Position of Body Parts—Various relative movements of parts of the animal's body can be sensed by devices that measure angles or distances. Thus, a jaw-angle sensor may consist of two rods, one connected to the case and one to the rotor of a miniature one-turn trimpot (Figure 16). With one rod sutured to the upper jaw and the other to the lower jaw, any opening or closing of the mouth will change the angle between the rods, thereby rotating the trimpot wiper. A similar sensor could measure the angle between the pectoral fins of a shark—a postural characteristic important in threat display. The flexing of a shark's clasper might also be sensed, giving data possibly related to sexual activity.

Body-position sensors would normally be attached at spots some distance from the main transmitter mounting, e.g., jaw sensor on jaw, main UST on back. Thus, an electrical cable must connect the two components unless a wireless method is devised. One such possibility is for the sensor unit itself to contain a tiny UST powerful enough to reach the main unit 1 or 2 m away. Received pulses would then be converted, based on their rate, into a variable resistance, perhaps by driving an LED/photocell vactrol.

Physiological Variables—Except for body temperature, most physiological parameters are not directly measurable by variable-resistive sensors of the general types described above. Physiological telemetry usually involves sensing or relaying voltage variations of one type or another; such techniques are discussed in detail by Mackay (1970). Although telemetry of internal physiological variables usually involves continuous transmission, certain
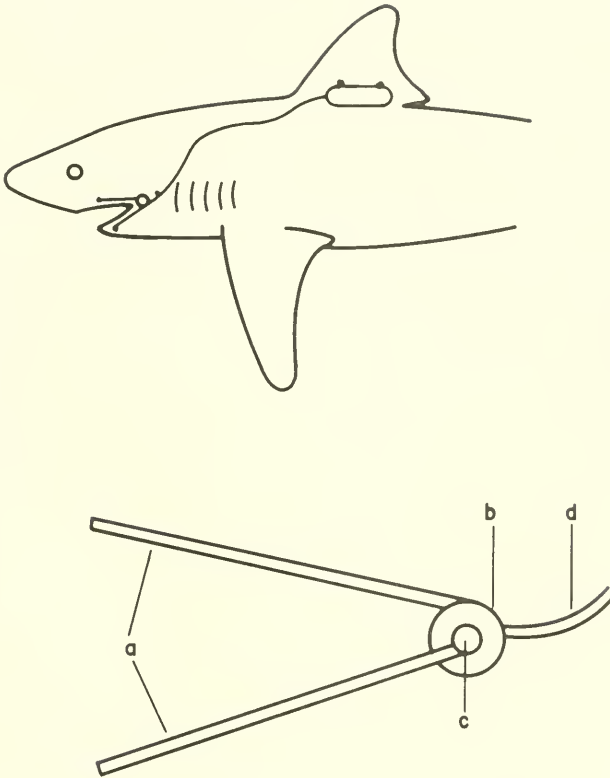
Figure 16 Body-position sensor for measuring jaw angle: (a) Rods sutured to skin. (b) Housing (attached to case of rotary trimpot). (c) O-ring-sealed shaft (attached to rotor of trimpot). (d) Cable to transmitter.

events such as heartbeats could be telemetered by pulsed transmission at long ranges. The main ECG spike as picked up by electrodes could directly trigger the production of a standard ultrasonic pulse thereby making transmitter pulse rate a direct duplication of heart rate.

Of special interest in shark behavior is the telemetry of pH or temperature from a stomach-implanted transmitter. Such data, in addition to its physiological implications, could provide indications of individual feeding events. When a piece of food and accompanying ocean water are swallowed, there should be a noticeable change in stomach pH and a possible change in stomach temperature, the latter especially in "warm-bodied" species.

Nonresistive Sensors—The sensors just described that were designed for use in the CSULB shark transmitters act as variable resistors, i.e., bulk resistors, passing current in either direction. The transmitter circuit was originally designed to use this type of sensor because, in multichannel applications, it is easier to multiplex sensors if they are all of one type. Since some desired sensors were already resistive types (thermistors, photocells), it was logical for the rest to be resistive.

However, various other sensor (transducer) types are available, such as those that vary capacitance, inductance, or voltage output. To use these in the CSULB shark transmitter would require additional circuitry. Other UST circuits have been designed with a particular sensor of this type in mind. Examples are the single-channel depth transmitters described by Luke et al. (1973) and Pincock and Luke (1975), which use strain-gauge (voltage output) pressure sensors. Many strain-gauge pressure transducers are commercially available, some being very small and accurate but possessing the disadvantages of relatively high cost and susceptibility to failure if exposed to more than two or three times design pressure.

Like transmitter circuits, sensors are often designed and built by the researchers themselves to fit particular needs. Mackay (1970) discusses methods of sensing various parameters of interest in biomedical telemetry. A review of transducers used for measurement purposes, including principles of operation, physical descriptions, and commercial suppliers, is given by Aronson (1974).

Application, Tracking, Recovery

Attachment to Shark—Several methods can be used for fastening the transmitter to the shark (Figure 17). The primary concern is to avoid excessive capture and application trauma or long-term irritation, which might affect data validity. The precise effects can only be surmised, but it would seem that gross capture and application trauma would considerably affect a shark's behavior immediately after release but that this effect would diminish with time and eventually disappear. Any irritation caused by the attached transmitter itself, however, probably would persist.

BACK
(BARBED DART)

FIN
(PINS OR SUTURES)

STOMACH
(SELF INGESTED
IN BAIT)

BODY CAVITY
(VIA INCISION)
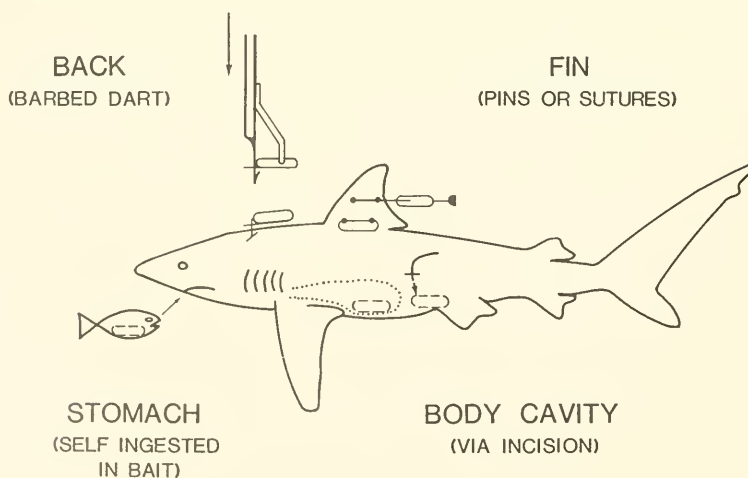
Figure 17 Transmitter application methods, external and internal. Application via barbed dart or by self-ingestion is accomplished without capturing the shark. The self-ingestion method is totally atraumatic.

For short-term trackings (hours, days) it seems best to use attachment methods that minimize initial trauma. Two proven methods usable on un-captured, free-swimming sharks are (1) external, via a barbed dart thrust in by an applicator pole, and (2) internal, by inducing the shark to swallow the transmitter concealed in bait. The latter method is totally atraumatic and does not appear to affect the shark's behavior at all. The former method usually results in the shark dashing away after being tagged. In the case of externally tagged blue sharks, however, normal behavior appeared to return within 60 to 90 min (Sciarrotta 1974). Some individuals seemed almost unaffected, circling back to the bait canister within seconds after applica-tion (Figure 1). On the other hand, some sharks captured and tagged after much struggling showed probably traumatic effects for many hours after release.

For longer term (weeks, months) studies it may be better to capture the shark and carefully attach the transmitter to its skin or fin in a way that minimizes longer term irritation. Stomach implantation eliminates irritation but expected lengths of retention are not yet well enough known, e.g., some sharks have regurgitated USTs within the first few days, another individual at 10 days. For long-term trackings the best method might be insertion of the transmitter in the body cavity via an abdominal incision.

Tracking—Tracking operations normally begin immediately after the unit is applied to the shark and are maintained either continuously or inter-mittently until the end of the mission, at which time an attempt is some-times made to recover the transmitter. The signal is detected using one of several tunable, narrow-band receivers, some of which are waterproof and designed for use in wet weather or by divers under water (Figure 18). When tracking from a small boat, the operator maintains signal contact using a directional hydrophone, the simplest method being manually holding the staff-mounted unit over the side of the drifting boat. It is much preferred, however, to utilize a streamlined gunwale or bow mount for the hydro-phone, as described by Stasko (1976), Stasko and Polar (1973), and Lawson and Carey (1972), which permits reception while the boat is under way at moderate speeds.

When reception is good (strong signal, low noise) an omnidirectional hydrophone can be used to maintain signal contact; this gives the trackers a rest from the tedium of manually maintaining proper aim of the directional hydrophone. When signal strength becomes marginal, its direction is deter-mined and the boat moved closer and again allowed to drift. Locations of the shark are plotted, and the telemetered data is stopwatch decoded or tape recorded for later computer reduction.

If the shark's location is determined relative to the boat, then the boat's location must be determined in order to plot the shark's true position. During the day, standard visual positioning methods usually suffice, using shoreline reference marks or special marker buoys deployed for this purpose. The primary problem occurs at night, especially in remote areas without lighted landmarks. Small-boat radar is one answer, if the shorelines are of

Figure 18   Tunable ultrasonic telemetry receivers designed for use either in boat or by divers underwater. Top: DuKane model N15A235A (DuKane Corp., Ultrasonics Div., St. Charles, Ill. 60174). Bottom: Burnett Model 512 (Burnett Electronics Lab., Inc., 7917 Balboa Ave., San Diego, Calif. 92111). Both units tune from 30 to 45 kHz.

sufficient target strength or if reference buoys are fitted with radar reflectors (better yet, radar transponders). Without radar, the reference buoys could carry underwater acoustic transmitters on frequencies different from that of the animal being tracked. Transponding units would allow fixing the buoys' positions from the boat. Timefix transmitters would be quite adequate also, as any needed periodic resynchronization would pose no problem with the surface buoys. Addition of a small light (flashlight bulb) to a buoy greatly facilitates locating it at night.

During good weather and good signal reception, tracking operations are relatively simple and straightforward. With bad weather, poor signal propagation, high ambient noise, etc., tracking can become difficult, and its success becomes dependent on the skill and determination of the tracking personnel. In certain coral-reef areas with strong currents, rugged bottom topography, and high ambient noise, it can be difficult to maintain unbroken contact with an actively moving shark, and trackings can include considerable time spent in searching and relocating.

Human Fatigue—Experience has shown that the main limitation to the length of a continuous tracking operation is fatigue of the tracking personnel. In studies of blue sharks at sea off California, 24 h has proven about the limit for continuous boat tracking by a crew of one or two persons (Sciarrotta 1974). At Rangiroa, French Polynesia, gray reef sharks were tracked nearly continuously for periods up to 96 h by a crew of four persons, each putting in one solo 6-h shift per day (Johnson and Nelson, unpublished data).

Considering this human problem, any hardware improvements that make tracking easier or permit more noncontinuous trackings are well worth developing. Manpower requirements should also be kept in mind during the planning of telemetry operations. It may be that long continuous trackings are not necessary to answer the biological question being asked. An intermittent schedule with adequate rest periods is certainly easier on the crew and may, in the final analysis, yield more useful information.

Fixed Receiver Arrays—An alternative to tracking from a single boat is to use an array of fixed hydrophone/receiver units distributed throughout the animal's home range. Such units would either record data for later examination or would be linked by cable or radio to a central receiving station for immediate availability. By comparison of pulse-arrival times at three or more omnidirectional units, relatively exact location fixes can be made, even from simple pingers. A system of this type was used by Hawkins et al. (1974) for tracking cod in a Scottish sea loch. Another example is the "pinpoint" system described by Holand et al. (1974) in which timefix USTs are automatically tracked and plotted on an xy-recorder using a multiple-hydrophone array and special microprocessor.

Complete area coverage by such a hydrophone array is practical only in relatively open environments with good acoustics. Hawkins et al. estimated that four hydrophones could cover an area of about 1 km^2. In the case of a shark ranging throughout a complex environment of coral reefs, passes, and

islands, many more hydrophones would be necessary for complete area coverage. For some study objectives, however, less than total coverage may suffice, e.g., detecting movements through passes or at specific feeding sites. A good example is Thorson's (1971) use of strategically placed shore monitors on the San Juan River to confirm movements of bull sharks between the Caribbean Sea and Lake Nicaragua.

A suggested system for tracking home-ranging reef sharks would use ultrasonic receiver buoys with radio-relay capability. These units, each on a separate radio frequency (perhaps CB channels), would be anchored at sites of interest in the shark's home range and would be monitored by radio at a nearby home base. Using a dual-trace storage oscilloscope, the operator would make the necessary pulse-arrival comparisons and plot the shark's position to the precision possible—depending on the number of hydrophones receiving the signal, the types of hydrophones, and the types of shark units involved (Table 1).

Omnidirectional hydrophones would be the simplest to use in radio-relay units but would yield relatively poor range because they take in noise from all directions. Directional hydrophones give better range but require a horizontal scanning mechanism if all directions are to be monitored. Such a scanning mechanism, e.g., a rotating reflector, could be continuously turning or radio controlled from the base station. The relayed signal must also be azimuth coded in some way. Although more complex, fewer such scanning units would be required as compared to nonscanning omnidirectional units. Nonscanning directional receivers can also be built on the principle of frequency-phase comparison among multiple hydrophone elements, where interelement distances are on the order of one wavelength.

If the shark unit is a timefix transmitter, locational fixes can be obtained from one directional relay unit or from two or three omnidirectional units (Table 1). Resynchronization of the timefix system can be accomplished whenever the shark comes into a position where its location can also be fixed by one of the nontimefix methods.

Transmitter Recovery—If desired, the transmitter package can include a timing device that releases the unit from the shark at the expected end of the tracking. Recovery is then accomplished under water by divers or from the boat if the package includes floatation. In the latter case, addition of a radio transmitter to the package provides additional recovery insurance, especially if the trackers had lost contact with the UST prior to release from the shark. Simple magnesium breakaway links (predictable corrosion rates) were used reliably for transmitter recoveries from angel and blue sharks (Standora 1972, Sciarrotta 1974), but detachment times varied considerably due to differences in water temperature and flow rate. This uncertainty of timing can be discouraging to weary tracking personnel.

A much more precise electronic release-timing mechanism is shown in Figure 19. A digital oscillator and counting circuit are set for a predetermined time, then an SCR switch closes to connect a battery across the seawater between an electrode plate and a thin breakaway wire. With about

Table 1.   Determination of transmitter position using measurement of pulse-arrival times.

Number of hydrophones	Omnidirectional hydrophone		Directional hydrophone	
	Pulse transit time un- known*	Pulse transit time known†	Pulse transit time un- known*	Pulse transit time known†
1	Presence only‡	Range only (on a circu- lar LOP)‖	Direction only (azimuthal LOP)	Fix (direc- tion and range)
2	On a hyper- bolic LOP	At one of two points (intersec- tions of circular LOPs)¶	Fix (inter- section of two direc- tions)	Fix
3	Fix (inter- section of hyperbolic LOPs)	Fix (inter- section of three circular LOPs)	Fix	Fix

*Pulse arrival times are known only relative to one another.
†Requires a system such as transponding or timefix.
‡Presence can be confirmed as within maximum transmitter range. Distance can be roughly estimated by signal strength, but this is subject to gross variability depending on propagation conditions.
‖LOP = line of position.
¶Often one of the two points can be ruled out, e.g., by previous fix location, by water depth, or by being on land.

350 mA of current flowing (from a 6-V battery), the 0.3-mm stainless steel wire deplates to the breaking point in about 60–90 s, causing the transmitter to eject from a base plate and float to the surface.

An alternative method is to use a tiny explosive squib to operate a transmitter-release mechanism. One such miniature explosive piston actuator (Holex, Inc., Hollister, CA) measures .53″ long, .135″ diameter, and fires in 15 msec with an applied current of 2.0 A (squib resistance, 0.65 ohm). This device can be fired by the circuit in Figure 19 if the SCR current-limiting resistor is removed.

The question of whether to plan for transmitter recovery is based on the tradeoff between the advantages of recovery and the disadvantages of what is needed to effect recovery. Advantages include savings in cost and time, added trackings possible with reuse of units, and the opportunity to inspect units that have been through actual trackings. Disadvantages include added
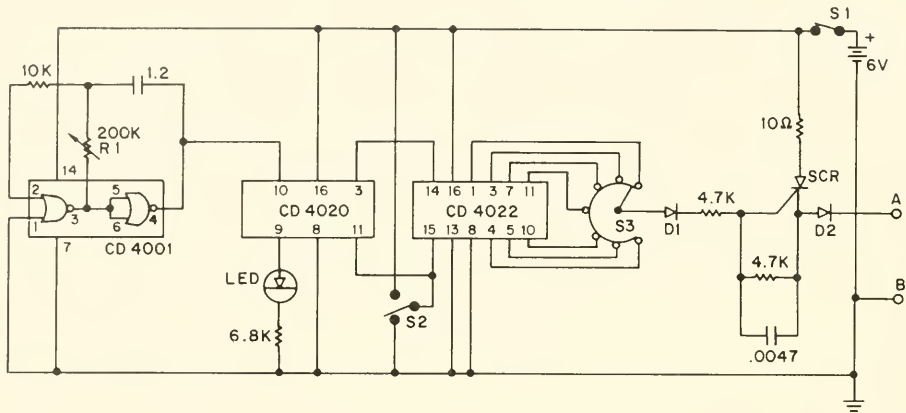
Figure 19 Circuit for a digital release-timing mechanism for detachment of transmitter at a predetermined time. The CMOS, R/C oscillator shown provides accuracies to approx. 20 s/day. If greater accuracies to within 1 s/day are required, a quartz-crystal oscillator may be substituted. Switch S3 provides (counter-clockwise from top) times of 1X, 3X, 5X, 7X, 9X, 11X, and 13X the input interval at pin 14 of the CD4022. Considerable time adjustment is available via R1; if this is sufficient, then CD4022 and S3 may be deleted and pin 3 of CD4020 connected directly to D1. Other counter combinations may be substituted if desired.

R1.   Frequency control for R/C oscillator
D1.   1N914
D2.   1N4001
SCR. 2N5060
LED. MV50. Provides visual indication of operation, can be omitted if desired. As shown, LED consumes about 0.8 mA.
S1.   Power switch
S2.   Counter-reset switch. To reset, momentarily throw to opposite position, then return to position shown.
S3.   Rotary time-selector switch
A.   Attachment point for breakaway wire (wire in seawater)
B.   Metal plate electrode (in seawater)

complexity, necessity to use attachments compatible with release, and greater package size and drag, especially if flotation is needed. In general, validity of data is the most important consideration, particularly for long-term trackings involving many days of boat and personnel time. If there is reason to suspect that a recovery-compatible attachment (e.g., external with float) would substantially affect the animal's behavior, then it would normally not be used. If, however, cost considerations severely limit the number of units available, the benefits to the whole study of repeated use of recovered transmitters might outweight the detriments of the recovery package.

Data Recording, Reduction, and Analysis

The simplest way to decode telemetered sensor data is to manually time the pulse rate with a stopwatch. This is easily done in the boat, and usually

suffices for trackings using one-channel transmitters. Multichannel data can also be field decoded in this manner if they are slow-multiplexed (Figure 5). Manual timing, however, is adequate only for pulse rates that are relatively slowly changing and cannot be used to measure rapid-multiplexed data or other kinds of rapidly changing pulse rates. A storage oscilloscope or recording paper oscillograph can be used to decode these kinds of data in the field.

The full potential of multichannel telemetry is realized only with rapid multiplexing, continuous tape recording of data, and subsequent computer reduction and analysis. The CSULB multichannel system was designed to detect brief, infrequent behavioral events as well as longer term changes. With all eight transmitter channels sampled within about 2 s (1 s if four channels used), even relatively brief events show up clearly in the data. For events likely to have durations of less than 1 or 2 s, the sensor should have a certain degree of "persistence" (see section on sensors).

Computer Processing—In the CSULB system, data from the receiver are first conditioned by pulse-amplitude and length-discriminating circuitry for initial noise rejection (Figure 20). The resulting uniform pulses are recorded, along with coded time-of-day information, at slow tape speed on an analog instrumentation recorder. Later these tapes are played at much higher

Figure 20 Apparatus for field recording of multichannel telemetry data prior to computer processing. The analog instrumentation recorder is fed from the pulse conditioner (black box). The telephone dial is for manual placement of time-of-day code on the tape (decimal code).

speeds into a PDP-12 computer[3] to digitize the pulse-interval times. The digital tape thus produced is then processed by the larger CDC-3150 computer for further noise reduction, demultiplexing, data averaging (when desired), and printout as eight parallel graphs of sensor state vs time of day (Figure 21).

Biological conclusions are drawn by visual inspection of the computer printouts. Averaged graphs would be scanned first, these including means and ranges for each averaging period. General trends over the day-night cycle would be apparent, and other points of interest would show up—possibly only as range excursions. These would be examined in greater detail on printouts at shorter averaging periods or on full-detail printouts.

In conjunction with a digital plotter, the computer can be programmed to draw maps showing detailed locomotor patterns of the telemetered sharks. If
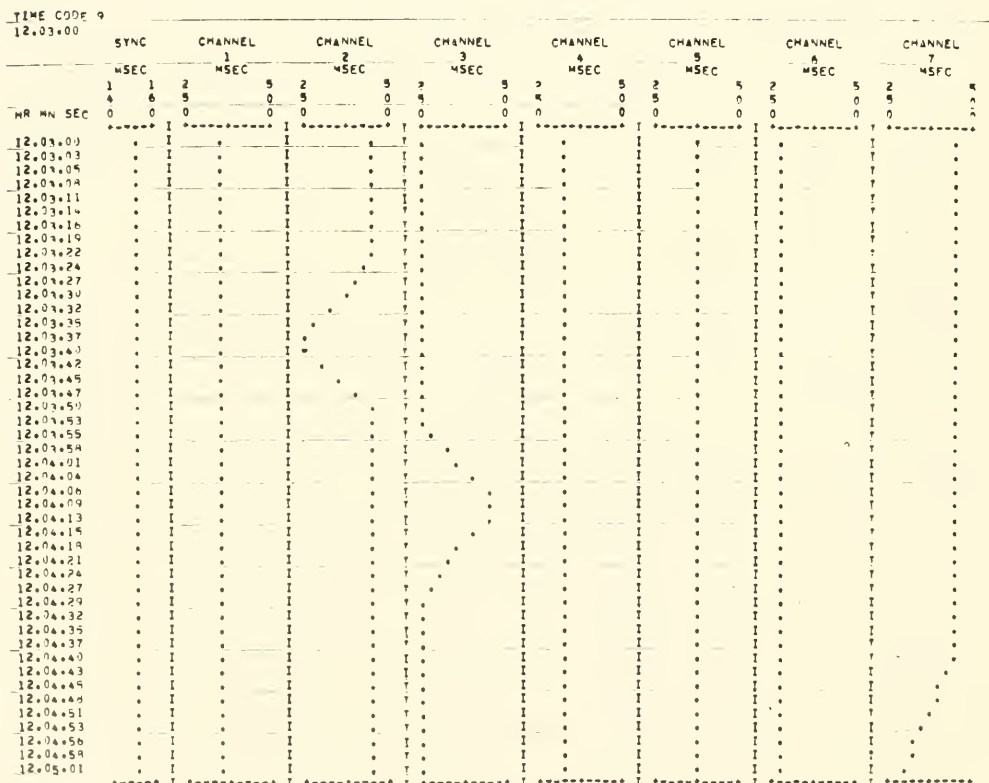
Figure 21 Full-detail computer printouts of simulated multichannel data (rapid multiplexed). Channels 2, 3, and 7 contain fluctuating data. The millisecond axis for each channel represents the pulse interval in that data frame for that sensor, i.e., changes in millisecond value indicate changes in sensor value. Times on the left margin are based on the time-of-day code (upper left) placed on the tape prior to each recording period.

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[3] An IMSAI 8080 microcomputer is now used for this operation.

combined data from several sensors (swim speed, compass, etc.) are used, such "micropath" plots can potentially yield a great amount of behavioral information. As a hypothetical example, a hunting shark might circle its prey closely, then accelerate to attack. Such a brief, small-scale pattern would be impossible to detect from the boat by hydrophone direction but would be readily apparent on the computer map.

System Performance—Range, Life, Size

When selecting a telemetry system for a particular research need, the trade-offs between transmitter range, life, and size must be considered. These three obviously important factors are interdependent and to emphasize one means, in effect, to sacrifice the others. Ideally, the compromise chosen should be based on the requirements of the usage in mind. Thus, maximizing range may be paramount when tracking a fast-swimming, wide-ranging pelagic species; to achieve this, a rather large, short-lived transmitter may be chosen. For monitoring a reef species with a limited, predictable home range, the investigator may choose much longer life at the sacrifice of some range. For relatively small species, range and/or life might have to be traded off to achieve an acceptably small transmitter size.

Transmitter range in the ocean depends on certain characteristics of the telemetry system and on various environmental factors, some quite variable and difficult to predict. *Absolute* range can thus only be estimated, and no dependence should be placed on such calculations before verification by in situ measurements under actual study conditions. It is useful, however, to consider the primary factors governing signal-detection range and how changes in these factors affect range, at least in a relative sense.

Ultrasonic Propagation in Seawater—Transmission of sound through water is a complex phenomenon, dependent on a multiplicity of factors, and has been treated in detail by Albers (1965), Urick (1967, 1975), and others (Eckart 1968). In regard to range estimates for USTs, *spreading* and *absorption* are the most important factors and the only ones for which simple calculations can be made.

Spreading (divergence)—As a sound pulse radiates outward from the source, its energy is spread over an increasingly larger area. In a medium without boundaries, this can be thought of as the surface of an expanding sphere. In relatively deep, open water, spreading is usually considered to be spherical, giving a decrease in sound level of 6 dB each time the distance from the source is doubled. In relatively shallow water with strong surface and bottom reflections, the diverging sound may become channeled, and a "modified cylindrical" spreading of between 3 and 6 dB per distance-doubling is sometimes appropriate (pure cylindrical spreading = 3 dB). Sound spreading is independent of frequency, temperature, and salinity but is affected by reflections, refractions, and losses at boundaries. Table 2 shows the relative transmission loss due to spherical spreading.

Table 2. Reduction in signal strength (sound pressure) due to spherical spreading only.

Distance from source (m)	Reduction (dB)	Distance from source (km)	Reduction (dB)
1	0	1	60
2	6	2	66
4	12	3	70
8	18	4	72
16	24	5	74
32	30	6	76
64	36	7	77
128	42	8	78
256	48	9	79
512	54	10	80

Absorption—As sound travels through the ocean, some of its energy is absorbed by the water itself and converted to heat. Unlike spreading, absorption varies with frequency, temperature, and salinity. To the UST user, the effect of frequency is of particular interest because it is a design parameter over which some choice can be exercised. Absorption at frequencies in the ultrasonic-telemetry range is listed in Table 3. At most UST frequencies, i.e., less than 100 kHz, absorption increases with decreasing temperature and increases with increasing salinity. Seawater, unfortunately, absorbs sound much more than does freshwater—from 10 to 30 times more than distilled water for frequencies in the UST range (Urick 1975).

Other factors—In addition to losses caused by spreading and absorption, some additional sound energy is usually lost due to a variety of difficult-to-calculate factors (scattering, refraction, etc.), sometimes collectively called *transmission anomalies*. Thus, losses calculated considering only spreading and absorption are likely to be less than those actually measured in the ocean. Furthermore, transmission losses measured from one spot to another are liable to fluctuate from day to day, hour to hour, and even second to second. Occasionally, losses turn out to be less than expected because of refractive or reflective channeling or constructive interferences, but that occurs only under special environmental circumstances (e.g., the deep sound channel) which are rarely of help in ultrasonic telemetry.

A diffuse reflection known as *scattering* can be a problem if the medium contains enough small particles with dimensions on the order of one wavelength or less, e.g., air bubbles, plankton, small fishes, and crustaceans. Such small scatterers can remove a significant amount of energy from the traveling

Table 3. Absorption of sound in seawater at 15°C.*

Frequency (kHz)	Absorption (dB/km)
10	0.60
20	2.2
30	4.9
40	8.8
50	12
60	16
80	24
100	33
150	49
200	60

*Values based on Fig. 5.4 of Urick (1975).

sound wave, one example being the concentration of organisms known as the deep scattering layer. Another example is the entrapment of air bubbles near the ocean's surface under conditions of breaking waves. These air bubbles can pose a serious tracking problem if both the telemetered animal and the receiving hydrophone are near the surface.

Downward *ray bending* can limit signal-detection range by the refractive effect of the vertical temperature gradient usually found in the ocean (temperature decreasing with depth). Thus, a transmitter near the surface may be undetectable beyond a certain distance by a shallow hydrophone, whereas a deeper hydrophone may still receive the signal.

Sound rays in the ultrasonic frequencies are also easily *blocked* by structures that are large with respect to one wavelength; they do not go around corners but instead form "sound shadows" behind such objects. Thus, if a large underwater structure intervenes between the transmitter and receiver, the signal can be severely reduced or blocked entirely. When tracking sharks in coral-reef areas, it is common for the tracker to hear a strong signal dwindle to weak or to nothing within a few seconds. This usually indicates that the shark has moved behind a reef. Sharks that enter coral caves present special problems for this reason, e.g., only a single narrow beam of sound may escape from the cave.

Ambient Noise—Background noise in the environment is one of the variables that make transmitter detection distance difficult to predict. Since under actual tracking conditions absolute signal level is seldom limiting, signal recognition is usually a matter of discriminating signal from noise, thus the noise level in the appropriate frequency band becomes an important

limiting factor. The classic curves of Knudsen et al. (1948) and Wenz (1962) present average spectrum-level values for ambient noise in the ocean. At the frequencies of ultrasonic telemetry, they plot noise values in terms of sea state or wind force; some of the pertinent values are reproduced in Table 4. While noise increases with increasing sea state, it decreases with increasing frequency, at a slope of approximately 5 to 6 dB/octave.

Tracking on reefs or inshore presents additional problems because of the preponderance of biological noises such as the "crackling" din of snapping shrimp (Fish 1964). While at a distance such sources may blend together into a relatively uniform background noise, at close range crustacean sounds are heard in the receiver individually as loud clicks. The problem then becomes one of discriminating such noise clicks from the transmitter pulses which may also sound like clicks if the pulse length is short. This is a different problem from that of trying to detect a faint transmitter pulse against relatively uniform background noise.

Thus, in noisy reef areas, range predictions based on the open-water noise values of Table 4 may be grossly optimistic. On the other hand, noise measurements made on the reef using "slow-response" noise meters (averaging times of several seconds) might give overly high values because of the relatively infrequent but intense sounds of nearby snapping shrimp. Such

Table 4. Average deep-water ambient-noise spectrum levels (dB re 1 μPa).[*†]

Frequency (kHz)	Ocean surface conditions								
	0	½	1	2	3	4	5	6	- Sea state
	0	1	2	3	4	5	6	7	- Beaufort wind force
	<1	1-3	4-6	7-10	11-16	17-21	22-27	28-33	-Windspeed (knots)
10	27	32	36	42	45	47	49	51	
20	22	27	31	37	40	42	44	46	
30	18	23	27	34	37	39	41	43	
40	17[‡]	21	26	33	35	37	39	41	
50	19[‡]	19[‡]	24	31	33	35	37	39	
60	20[‡]	20[‡]	23	29	32	34	36	38	
80	23[‡]	23[‡]	23[‡]	27	30	32	34	36	
100	25[‡]	25[‡]	25[‡]	25[‡]	28	30	32	34	

[*]Spectrum-level noise is defined as the amount of noise in a frequency bandwidth of 1 Hz. Sound pressure expressed as decibels relative to 1 micropascal (0 dB re 1 μPa = –100 dB re 1 microbar).

[†]Data adapted from Figure 7.5 of Urick (1975). These values are within about 2-3 dB of those given by Knudsen et al. (1948) and Wenz (1962). Values for shallow-water/- coastal areas may be higher by 5-10 dB or more, and are generally more variable.

[‡]Noise due to molecular agitation (thermal noise).

high-level spikes can drive the meter reading up to a point from which it cannot subside sufficiently in the relatively quiet periods in between. When tracking in reef areas, it is generally preferable to position the boat so that the hydrophone need not be pointed in the general direction of noisy reef surfaces that are much closer than the transmitter being tracked.

Transmitter Characteristics

Frequency—It is evident from Tables 2 and 3 that at very short distances significant transmission losses are almost entirely due to spreading, whereas at relatively long distances the losses become increasingly due to absorption (which is frequency dependent). From this, it follows that, if relatively long ranges in seawater are desired, frequency becomes a critical parameter and it becomes more important to use a lower frequency. Considering that the higher ambient noise at lower frequencies partially negates the lesser absorption at lower frequencies, it is possible to calculate for any given distance which frequency will theoretically reach that distance most efficiently. From Figure 22 it can be seen that, if a maximum range of only 200 m is desired, the optimum frequency is much higher than if a range of 2 km is attempted.

Output power—Since transmitter range is partly a function of transmitter signal strength, the question arises as to how much electrical power is required to achieve a given sound pressure level (SPL). The significant factors are (1) the efficiency of the transmitter power-amplifier section in converting battery power into higher voltage power at the input to
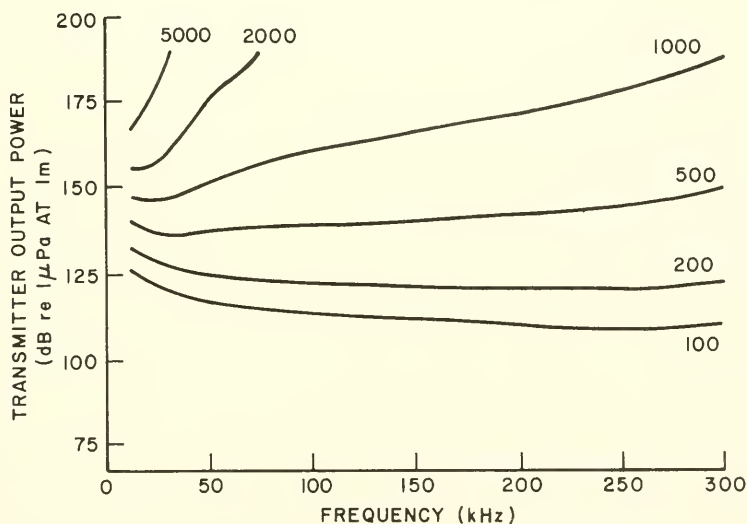
Figure 22   Transmitter strengths required to achieve various ranges in coastal sea water (sea state 3) as a function of frequency. For a signal-to-noise ratio of 0 dB (RD = 0), a receiver bandwidth of 1 kHz, and a moderately directional hydrophone. Redrawn from Stasko and Pincock (1977).

the PZT transducer, (2) the efficiency of the PZT transducer in converting electrical power into acoustic power, and (3) the directivity index (DI) of the PZT element in radiating sound outward (see section on *Hydrophone directivity*).

The following relationships obtain:

1 electrical watt = 1 acoustical watt (if energy conversion is 100% efficient).

1 acoustical watt radiated omnidirectionally (spherical spreading) = a signal strength (SPL) of +170.8 dB re 1 μPa at 1 m distance (absorption can be neglected at this short distance).

For example, the CSULB Mark V shark transmitter draws approximately 500 mA at 8 V (= 4 electrical watts) from the battery during the power pulse. If amplifier efficiency and transducer efficiency are each assumed to be 50%, then 4 W from battery = 2 W into PZT = 1 acoustical watt into the water. For the broadly toroidal radiation pattern produced by the thin-walled PZT cylinder, the directivity index can be considered about 1.5 dB (for full omnidirectionality, DI = 0 dB).

The following equation may then be used:

$$
\begin{aligned}
\text{SPL at 1 m} &= 170.8 + 10 \log \text{acoustic power} + \text{DI} \\
&= 170.8 + 10\,(\log 1.0) + 1.5 \\
&= 170.8 + 0 + 1.5 \\
&= 172.3 \text{ dB re 1 } \mu\text{Pa (in the radial direction)}^4
\end{aligned}
\tag{1}
$$

This calculated value of 172.3 dB re 1 μPa is close to the measured value[5] of 171.8 dB re 1 μPa, indicating that the efficiency estimations of 50% were about correct.

Pulse length—It is the general experience of tracking personnel that increased pulse length (pulse width) results in increased signal-recognition range, at least up to lengths of roughly 20 ms. Reasons for this are complex and varied but partly involve the greater amount of energy in the longer pulses and the lesser chance that all or most of this energy will be lost due to transmission anomalies such as time stretching and fluctuating, multipath-induced interferences. Another reason is that short pulses sound more like clicks and are thus more difficult to distinguish from other short ambient-noise clicks, such as those produced by snapping shrimp. As pulse length increases to more than about 5 ms, the listener becomes increasingly aware of the tonal (frequency) quality of the pulse, and this aids considerably in recognition. Still another reason involves the limitations set by the slow response time of narrowband receivers (see section on Receiver Characteristics).

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[4]dBμbar = decibels re 1 dyne/cm^2 = dB re 1 μPa - 100.

[5]Sound level measurement courtesy of International Transducer Corp., Goleta, Calif.

Pulse rate—When pulse rates are slowed to less than about 1/s, tracking also becomes increasingly difficult, especially in noisy areas. Tracking personnel distinguish transmitter pulses from noise pulses mainly on the basis of the repetitive pattern in which they occur. Hearing just one or two pulses does not confirm signal presence, except for pulses long enough to possess an unmistakable tonal quality. The tracker requires at least several successive pulses at the expected rate before recognition can be confirmed. At excessively slow pulse rates, the listening time needed for recognition becomes too long. This can be a serious disadvantage in reef areas where transmission is often interrupted as the animal moves behind signal-blocking structures. Similarly, a slow pulse rate necessitates a slow rate of scanning with the directional hydrophone so that it takes much longer to complete a 360° scan of the underwater horizon. In general, pingers or single-channel USTs are easier to recognize than multiplexed units with more irregular pulse intervals. With the latter, it is even more important that pulse intervals not be excessively long.

Receiver Characteristics—When considering the effect of the receiver/ hydrophone system on theoretical signal-detection range, the following characteristics are pertinent.

Absolute sensitivity—For a signal in the receiver headphones to be recognized by the tracker, it must be present at the receiver input above a certain absolute level; otherwise it will be lost in the receiver's internal noise. This value is usually called the *receiver sensitivity* or *receiver noise level* and is specified in microvolts, e.g., the DuKane N15A235A receiver noise level is given as 0.1 μV equivalent at input. Thus, to be recognized, the electrical signal from the hydrophone must be somewhat above 0.1 μV—no matter how low the environmental noise might be.

This shifts the question to what level of acoustic signal at the hydrophone is needed to produce the required 0.1 μV electrical output. Hydrophone sensitivity is usually given in dB re 1 V/μPa, i.e., the voltage (in terms of dB below 1 V) that results from a sound pressure of 1 μPa. Again, using the DuKane receiver as an example, its hydrophone is specified as being a minimum of −185 dB re 1 V/μPa. From Table 5, it is seen that 0.1 μV is equivalent to −140 dB re 1 V. Therefore, any acoustic signal of less than 45 dB re 1 μPa cannot be detected because it will not produce the required 0.1 μV at the receiver input (45 dB above −185 dB = −140 dB).

Bandwidth—An important characteristic of the receiver/hydrophone system is the effective frequency bandwidth, usually defined as the total width (in hertz) to the half-power points (3 dB down) on both sides of the peak of the frequency-response curve. For the DuKane receiver, bandwidth to the −3 dB points appears to be considerably less than 1 kHz (±500 Hz).[6]

In general, a relatively narrow band receiver is preferable because the narrower the bandwidth, the more the ambient noise that is excluded. This

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[6]Specifications state a 1-kHz bandwidth to the −10 dB points.

Table 5. Electrical signal
levels expressed as dB rel-
ative to 1 V.

Voltage	dB re 1 V
1 V	0
100 mV	−20
10 mV	−40
1 mV	−60
100 μV	−80
10 μV	−100
3 μV	−110
1 μV	−120
0.3 μV	−130
0.1 μV	−140

gives a better signal-to-noise performance and therefore a greater signal-
detection range. However, if the bandwidth is too narrow, certain problems
can arise. One is that missing the signal becomes more likely if the tuning
dial is set just slightly off the precise frequency required. A second problem
involves the time it takes the relatively high-Q circuit (Q = center frequency/
bandwidth) to resonate up to full amplitude in response to an incoming
signal. The narrower the bandwidth, the longer it takes the circuit to respond
fully, and this puts a practical limit on the shortness of pulse that can be
effectively received. As a general rule for a 1-kHz bandwidth, the signal pulse
(tone burst) should be no shorter than 1 ms; for 500-Hz bandwidth, 2 ms;
for 100-Hz bandwidth, 10 ms, etc. This effect is important when choosing
transmitter pulse length, especially if one is considering reducing pulse length
in an effort to extend battery life. A third problem with some narrowband
receivers is that they are prone to "ringing" if hit with a high-energy pulse,
even a very short one, such as from a nearby snapping shrimp. Such a shrimp
click can then sound longer and more "tonal" than it really is, making it
more difficult to discriminate from a transmitter pulse.

Hydrophone directivity—Another important noise-reducing charac-
teristic is the degree of directionality of the receiver hydrophone. The
beamwidth is usually defined as the total angle to both the right and left of
the beam axis to where the response drops to the −3 dB points. For the
DuKane hydrophone, this is about 30° (15° on each side of axis). The effect
of beamwidth on total noise reduction is given by the *directivity index*,
which expresses in decibels the relative improvement in signal-to-noise ratio
of a given beamwidth as compared to omnidirectionality. Table 6 gives some
representative values of directivity index for conical beam patterns and
shows that the 30° DuKane beam yields a DI of about 16 dB.

Table 6. Directivity indexes for various beamwidths (to −3 dB points) of conical beam patterns.

Beamwidth (deg)	Directivity index (dB)
10	26
20	20
30	16
45	13
60	10
90	7
180	3
360 (omni)	0

Signal-Detection Level—Assuming that an acoustic signal arrives at the hydrophone at a level high enough that absolute receiver sensitivity is not limiting, the next question involves whether it can be recognized above ambient noise in the environment. The signal strength in dB re 1 μPa necessary for recognition is called the minimum detectable signal (MDS) and is given by the equation

$$MDS = Ns + 10 \log BW - DI + RD, \tag{2}$$

where Ns is the spectrum-level noise in dB re 1 μPa at the frequency of the signal, BW is the receiver bandwidth in hertz, DI is the directivity index of the receiving hydrophone in decibels, and RD is the recognition differential in decibels.

The *recognition differential* is a measure of how far the signal must be above noise at the receiver output in order to be recognized. For human listeners, the RD can be considered to be in the area of 0 to 3 dB, the human ear being quite good at signal discrimination. This value partly depends on the temporal nature of the noise. For example, a slow hammering on a metal plate would sound very loud (and would produce a high reading on a "slow-averaging" sound level meter), yet the faint sound of a telephone ringing would still be audible in the periods between the hammer strikes. Thus, the RD is really meant to indicate a signal-to-noise ratio more or less just during the signal pulse. For nonhuman "listeners," such as the automatic signal-recognition circuits of transponders, the RD may be considerably higher, e.g., 10–15 dB for some simple systems.

As an example, consider the following calculation for a signal of 40 kHz, the DuKane receiver (BW estimated 500 Hz, DI = 16 dB), a sea state of 2 (Ns = 33 dB re 1 μPa + estimated 5 dB for coastal conditions = 38 dB re 1 μPa), and an RD of 3 dB:

$$\begin{aligned} \text{MDS} &= 38 + 10 \log 500 - 16 + 3 \\ &= 38 + 10\,(2.7) - 16 + 3 \\ &= 38 + 27 - 16 + 3 \\ &= 52 \text{ dB re 1 } \mu\text{Pa.} \end{aligned}$$

Therefore, since the receiver absolute sensitivity limit of 45 dB re 1 μPa is lower, the effective MDS will be the 52 dB re 1 μPa value as calculated above.

Signal-Detection Range—If the above MDS value is used, a theoretical range can be calculated for any given transmitter by starting with its known signal strength and applying the estimated transmission losses. This can be done in tabular form, as shown in Table 7 for the Mark V shark transmitters, which emit 40-kHz pulses at a level of about + 172 dB re 1 μPa at 1 m.

Thus, according to Table 7, the transmitter's signal will diminish to the MDS level of 52 dB re 1 μPa at approximately 5 km. However, since Table 7

Table 7. Diminution of signal strength with distance, considering losses due only to spreading and absorption, for a transmitter source level of + 172 dB re 1 μPa at 1 m.

Distance from transmitter (km)	Spherical spreading loss (dB)	Absorption loss at 40 kHz (dB)*	Total transmission loss (dB)	Signal strength (dB re 1 μ Pa)
0.001	0	0	0	172
0.01	20	0.1	20	152
0.1	40	0.9	41	131
1.0	60	9	69	103
2	66	18	84	88
3	70	27	97	75
4	72	36	108	64
5	74	45	119	53
6	76	54	130	42
7	77	63	140	32
8	78	72	150	22
9	79	81	160	12
10	80	90	170	2

*From Table 3.

neglects losses from the more unpredictable factors, such as scattering and refraction, the actual transmitter range will probably be less. Even shorter ranges would be expected at higher ambient noise levels, or when the receiver is not in the optimum radial direction from the transducer element of the transmitter. It must be kept in mind that the previous discussion is intended only as a simplified introduction to the kinds of numbers involved in calculations of range estimates and should not be regarded as a method for precise prediction. Before any reliance is placed on calculated estimates, in situ verifications should be made. Range calculations confirmed by actual measurements in coastal seawater are graphed by Stasko and Pincock (1977) for various UST frequencies (Figure 22).

   Transmitter Size, Life—Although much of the transmitter circuitry can be miniaturized to very small dimensions, overall package smallness is still very much limited by battery size and, to a certain extent, by the diameter of the acoustic transducer element.

   Transducer size—The frequency of choice can place one limit on overall size because the resonant frequencies of the normally used PZT cylinders vary linearly with diameter as shown in Table 8. Cylinder length has relatively little effect; most elements used in USTs have length-to-diameter ratios in the range of about 0.5 to 2.0.
   If the PZT cylinder is bonded rigidly to the inside of the transmitter housing, the resonant frequency is lowered somewhat. For the air-filled Mark V shark transmitter, a 40-kHz resonance is achieved with a 22.2-mm (0.875 in.) OD element epoxied to the inside of the rigid-PVC transmitter housing.

   Batteries—Usually the major factor limiting transmitter size reduction is the battery capacity required for the desired range and life. The most relevant characteristic is energy density/unit weight, but other factors are usually considered, such as cost, shelf life, initial voltage per cell, voltage

Table 8. In-water resonant frequencies (circumferential mode) of air-backed, thin-walled cylinders of PZT-4 (lead zirconate-titanate).

Outside diameter (in.)	Resonant frequency (kHz)
0.25 (6.35 mm)	160
0.50 (12.7 mm)	80
1.0 (25.4 mm)	40
2.0 (50.8 mm)	20
4.0 (101.6 mm)	10

drop during discharge, environmental effects, and the availability of desired sizes and shapes. The commonly used mercury and silver-oxide cells have been standard in biotelemetric applications, but the recently developed lithium cells appear superior in many respects and may become the standard in the future, especially in applications in which battery cost is not limiting. There seems little reason to consider other battery types for UST use. The common carbon-zinc and alkaline cells, while inexpensive, are poorer in both energy density and voltage stability. A comparison of some primary (nonrechargeable) batteries is given in the following paragraphs, in Table 9, and by Heller (1975).

Mercury (mercuric oxide): Frequently used for UST applications because of its compactness, moderately flat voltage discharge curve, and moderate cost. Readily available from several manufacturers in a large variety of sizes and shapes, including series-wired stacks for higher voltages.

Silver oxide: About the same energy density as mercury but with a higher voltage and a flatter voltage discharge curve. Made only in small sizes (up to 160 mAh), therefore not convenient for the main battery for higher powered USTs. Useful for powering sections of circuits where quite stable voltage is needed.

Lithium: A major improvement over mercury or silver oxide in terms of energy density, especially per unit weight. These cells also possess very flat voltage discharge curves. Lithium-battery chemistry is of two distinct types, the organic-electrolyte type and the inorganic-electrolyte type. At this writing, the former is more readily available and less costly per cell, but the

Table 9. Performance characteristics of some nonrechargeable types of batteries.*

Battery type	Nominal voltage per cell (V)	Cutoff voltage (end of life) (V)	Energy density (W·h/lb)	Energy density (W·h/cu. in.)	Voltage stability during discharge
Carbon zinc	1.5	0.7	30	2	Poor
Alkaline	1.5	0.8	35	3	Poor
Mercury	1.35–1.4	0.9	45–58	7	Good
Silver oxide	1.5	1.0	38–48	7	Very good
Lithium (organic)	2.8–2.9	1.8–2.0	95–150	8	Very good to excellent
Lithium (inorganic)	3.6–3.9	3.0^+	220–250	15	Excellent

*Data from Heller (1975) and various battery manufacturer specifications. The ranges of voltages and energy densities are due to variations in manufacturer, specific chemistry involved, and size and shape of cell.

latter is superior in voltage and energy density. While superficially appearing to cost several times more than mercury cells, if compared on a cost-per-watt-hour basis, lithium cells appear reasonable and in some cases even less expensive than mercury. One present problem with lithium is the lack of availability of sufficient sizes and shapes, most being large, elongate cells such as AA, C, or D sizes.

Duty cycle—In a pulsed UST, the duty cycle is normally defined as the percentage of total time that the power-output stage is on, e.g., 10-ms pulses at a rate of 2/s give a 2% duty cycle. Battery life, however, depends on an "average" current drain consisting of a combination of (1) brief, heavy drains during the pulses, often partially "smoothed" by a large capacitor across the battery, and (2) continuous low-level drains of those circuit sections that are continuously on. In most transmitters, the first of these factors is the most significant. The obvious strategy of reducing the duty cycle to conserve the battery, unfortunately, can be carried only so far. As discussed earlier, there are limits for both pulse shortness and pulse-rate slowness beyond which signal detection or recognition is impaired.

Transmitter life—Battery capacity is usually specified as the number of milliampere hours that can be produced before the voltage drops to a certain arbitrary cutoff value, e.g., 0.9 V for mercury cells. Battery life in a UST can therefore be approximated by dividing the capacity (in milliampere-hours) by the average current drain (in milliamperes). It should be realized that, since voltage drops with battery use, the current drawn also drops; this must be considered when calculating average current. Furthermore, the manufacturer's specified capacity is valid only if the battery is used at the specified temperature and constant discharge rate. Under the extremely varying (pulsed) drain conditions of low duty cycle USTs, the specified capacities may or may not be applicable. Thus, the best estimates of transmitter life are those based on test runs in which a battery is consumed actually operating the transmitter. Tests on the CSULB shark transmitters at 2–3% duty cycles indicate that actual life is reasonably close to manufacturer's specified life for 600- and 750-mAh, 8.4-V mercury batteries.

It is useful to consider how relative changes in range or life affect needed battery capacity. Consider spherical spreading only; to double range, acoustic output must be raised by 6 dB, and this requires a fourfold (4X) boost in electrical power, thus four times the battery capacity for equivalent life. But since at any significant distance absorption must also be considered, it will actually require much more than a 4X battery increase to double range without reducing life. For instance, Table 7 shows that to boost range from 1 km to 2 km at 40 kHz actually requires a 15-dB signal increase, and this means a 32X battery boost. On the other hand, to double life without changing range requires only a twofold battery increase, to quadruple life means a fourfold battery boost, etc.

Thus, at the usual distances involved in shark telemetry, increased life is relatively easier to achieve than increased range. One can pay dearly in battery drain in attempting to gain "long" range by boosting output power

alone. A more efficient method would be to lower frequency and/or improve the receiving system.

RADIO TECHNIQUES

Long-range radio tracking of terrestrial animals is now a widely used technique with an extensive literature (Mackay 1970, Long 1977). As mentioned earlier, radio-tracking techniques have also been used successfully on certain freshwater species in relatively shallow water (Winter et al. 1973, Monan et al. 1975). However, radio techniques presently hold little promise for the direct tracking of saltwater species. The reason is the severe attenuation of radio signals as they propagate through a conducting medium. According to Mackay (1970), radio attenuation in seawater is approximately 55 dB/wavelength. Furthermore, for a given frequency, electromagnetic wavelengths in seawater are much shorter than in air due to a reduction in velocity of propagation, e.g., 1 MHz wavelength = 1.8 m, 150 MHz = 0.15 m. Thus, at the distances and depths traversed by free-ranging sharks, radio telemetry from the animal *through the water* appears out of the question, and ultrasonic telemetry remains the only practical method.

However, not all questions concerning the movements of sharks need be investigated by continuous trackings. If one is interested in general patterns over considerable periods of time, it may suffice to obtain several points along the movement route. Such data could be provided by one or more small radio transmitters, which, at predetermined times, detach from the animal and float to the surface, from where they could be detected through the air at long ranges. The simplest example would be single-radio trackings providing two data points (application site, popup site), but more data points could be obtained using multiple-radio packages with staggered release times, e.g., 24, 48, 72 h, etc.

Timed-Release, Floating Radio Transmitter

Following the previous reasoning, a pilot study using floating radio transmitters has been started at CSULB, to determine movements of pelagic sharks in situations in which ultrasonic trackings are logistically impractical. One main advantage of the radio method is the great saving in manhours spent tracking. While 24-h sonic tracking requires constant vigilance for the whole time, 24-h radio operation requires attention for only the initial application time, then a short time the next day to determine signal location and (if practical) make the recovery. While one crew would be entirely occupied with a single sonic tracking, it could handle radio trackings for a number of different sharks on the same day.

The prototype transmitter package shown in Figure 23 uses a standard "off-the-shelf," two-stage, 151-mHz Wildlife transmitter[7] and a 30-cm

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[7]Manufactured by Wildlife Materials, Inc., Route 3, Carbondale, Ill. 62901.
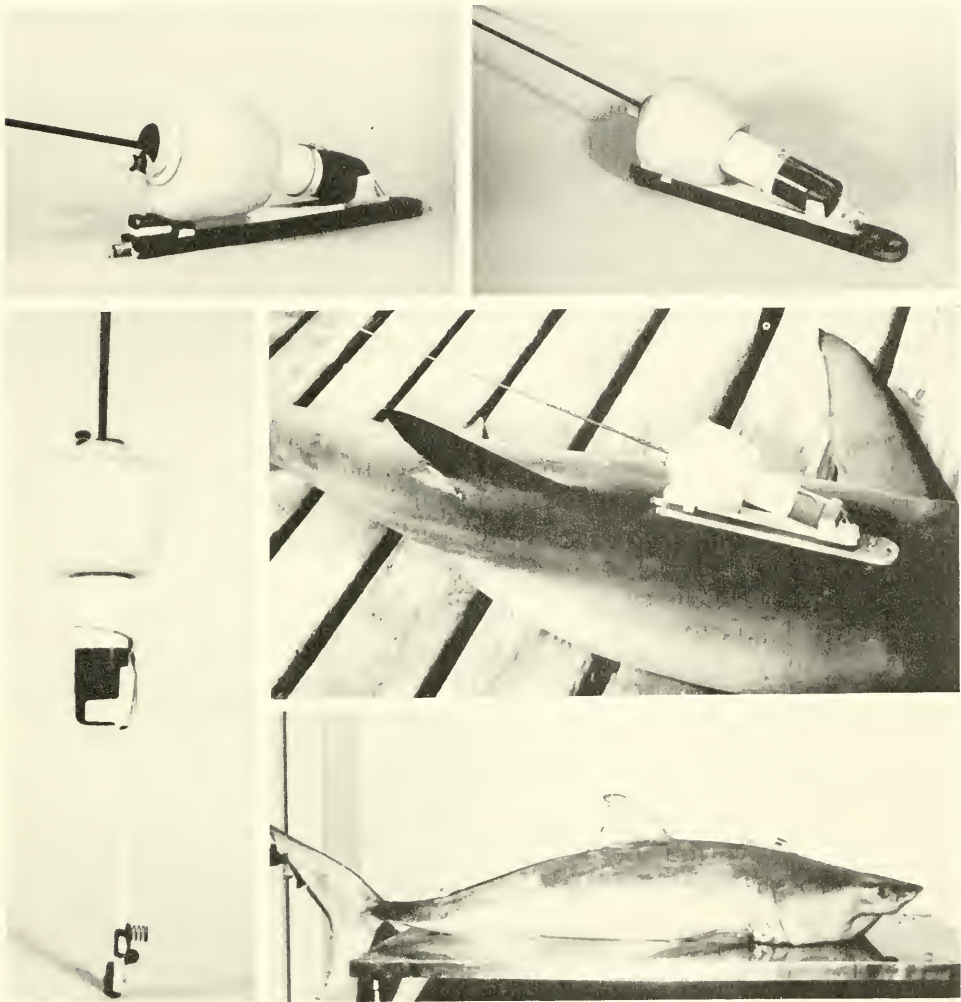
Figure 23 A prototype timed-release, radio-float transmitter designed for application to free-swimming sharks. Upper photos: Unit in folded position attached to expendable baseplate, showing breakaway wire and electrode plate. Lower left: After separation from baseplate and unfolding of counterweight arm. Center right: Demonstrated on a 1.7-m blue shark. Lower right: Demonstrated on a 2.2-m white shark.

vertical fiberglass-covered whip antenna. The unit is set for a rather rapid pulse rate of 4/s to facilitate recognition of the signal under conditions of intermittent reception, e.g., signals blocked when unit is in trough of waves. Length of the continuous-wave (CW) pulses is about 50 ms, and the current drain during the pulse is about 1.5 mA. The 3V, 160-mAh mercury battery will run the unit continuously for about 10 days. For popup times of several days or less, therefore, the radio may be simply turned on prior to application, remaining on until recovery. For longer durations, a mercury switch

(tilt switch) may be incorporated to activate the radio when the package detaches from the shark and assumes an upright position.

The transmitter housing includes an accurate digital timer and release circuit that detaches the unit from the shark (Figure 19). At the predetermined time, an SCR switch closes, and battery current flows through the breakaway wire, deplating it to the breaking point within a minute or so. Upon link breakage, the package separates from its expendable baseplate, unfolds a counterweight arm, and floats to the surface where it maintains an upright position with the antenna vertical (Figure 23).

An additional use for this radio system is to ensure recovery of ultrasonic telemetry packages in case sonic contact is lost during tracking. A timed-release, multichannel UST fitted with a radio beacon is shown in Figure 24.

Data recording—Although the radio units previously described are location-only transmitters, devices may be included in the recoverable packages which would record sensor data during the entire period between attachment and release, e.g., depths vs time. See previous section on data storage transmitters.

Figure 24 A multichannel ultrasonic transmitter (twin-tube type) fitted with a radio beacon to facilitate location after detachment from a shark. Demonstrated attached to the dorsal fin of a 2.2-m white shark.

Receiver/Antenna System

A standard terrestrial-wildlife tracking system is utilized, in this case an AVM model LA-12 receiver (12 frequency channels),[8] and a hand-held yagi directional antenna (3-element, 112-cm long) (Figure 25). The higher gain, 4.3-m, 8-element yagi provides maximum range under conditions where it can be handled, e.g., larger boat, land-based vehicle. Best directionality is obtained by using a null-peak, twin yagi or loop antenna system.

For relatively short-range reception, such as from a boat, a hand-held or mast-mounted antenna suffices. For maximum range detection, the antenna should be elevated higher than is practical from a small boat; airplane tracking is desirable. Another method to achieve long ranges is to use receiving stations on land at high elevations, such as on the mountains of Santa Catalina Island, Calif. The ultimate tracking system would involve satellites, the feasibility of which has been investigated by Goodman et al. (1973) for both marine and terrestrial species.

Signal-Reception Range

Under conditions of good propagation, a radio signal in air diminishes in strength with distance primarily because of spreading. There is nothing comparable to the large through-water absorptive losses of ultrasonic transmission, thus ranges obtained by radio units through air are much greater than those of USTs of the same power. Once in the far field of the radiating antenna (beyond about one wavelength), the field strength can be considered to diminish as the inverse first power of the distance, i.e., spherical spreading at 6 dB/double distance. Thus, a radio signal 1 μV/m at 1 km should diminish only to 0.5 μV/m at 2 km and so on.

In practice, signal ranges over water depend strongly on the height of the receiving antenna. Generally, the higher the antenna, the greater the range, unless a phenomenon called ducting occurs (Mackay 1970); it can result in unexpectedly long ranges near the water's surface. For the higher frequencies such as 151 MHz (which are virtually line-of-sight), the earth's curvature is mainly responsible for the need to raise the antenna at long ranges. A general indication of the range capabilities of transmitters of the types discussed are provided in Tables 10 and 11.

APPLICATIONS IN BIOLOGICAL STUDIES

A complex telemetry system is justifiable for a biological research program only if it can yield the desired biological information better than other, less costly methods. Telemetry is particularly applicable to gathering behavioral and physiological information on certain relatively large animals that are both difficult to observe in the wild and difficult or impossible to keep in captivity. Such is the case with many species of large, wide-ranging sharks.

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[8] AVM Instrument Co., 810 Dennison Dr., Champaign, Ill. 61820.

Figure 25   Radio receiving system for the transmitters shown in Figures 23 and 24. Top right: Reception from an elevated site on Santa Catalina Island. Left: Reception from small boat at sea, using elevated, hand-held antenna. System shown is the AVM model LA-12 receiver with the small yagi directional antenna. Initial tests with the prototype shark transmitter indicated ranges of 24–40 km from the elevated island site (70 m above sea level).

A discussion of some of the applications of telemetry in studies of free-ranging sharks follows. Much of the kinds of data thus obtained would be difficult or impossible to obtain by other methods.

Table 10. Signal-reception ranges over water for a 140-MHz transmitter of 1-mW power with a 50-cm (quarterwave) vertical whip antenna. Unit transmitting from the surface of the ocean (sea state 0).*

Receiver altitude (m)	Range (km)
15	6+
150	20
300	32
600	45
900	57
1200	70
1500	80

*From Schevill and Watkins (1966), cited in Mackay (1970).

Table 11. Typical ranges over land for 151-MHz transmitters of about 1 mW power with 30-cm vertical whip antennas.*

Receiving situation (transmitter on ground)	Range (km)
Hand-held antenna (small yagi)	3.7
Vehicle-mounted antenna (large yagi)	4.8
Mast-mounted antenna	
(9 m high)	7.2
(15 m high)	9.6
(30 m high)	14
Antenna on aircraft	
(600–900 m high)	56

*Data from AVM Instrument Co.

Daily Movement, Home Range

The most basic information obtained from telemetry of a free-ranging animal is the individual's location throughout the tracking, from which its home range, day-night movements, and so forth, can be plotted as was done by Standora (1972) for the Pacific angel shark (Figure 26). This is the original purpose of ultrasonic fish tracking, and it can be accomplished with simple pingers without sensors, which yield approximate location as a function of signal strength and direction. Better plots can be obtained, however, by using one of the systems more suitable to accurate location fixing, e.g., transponding, timefix, or multiple-receiver arrays (Table 1).
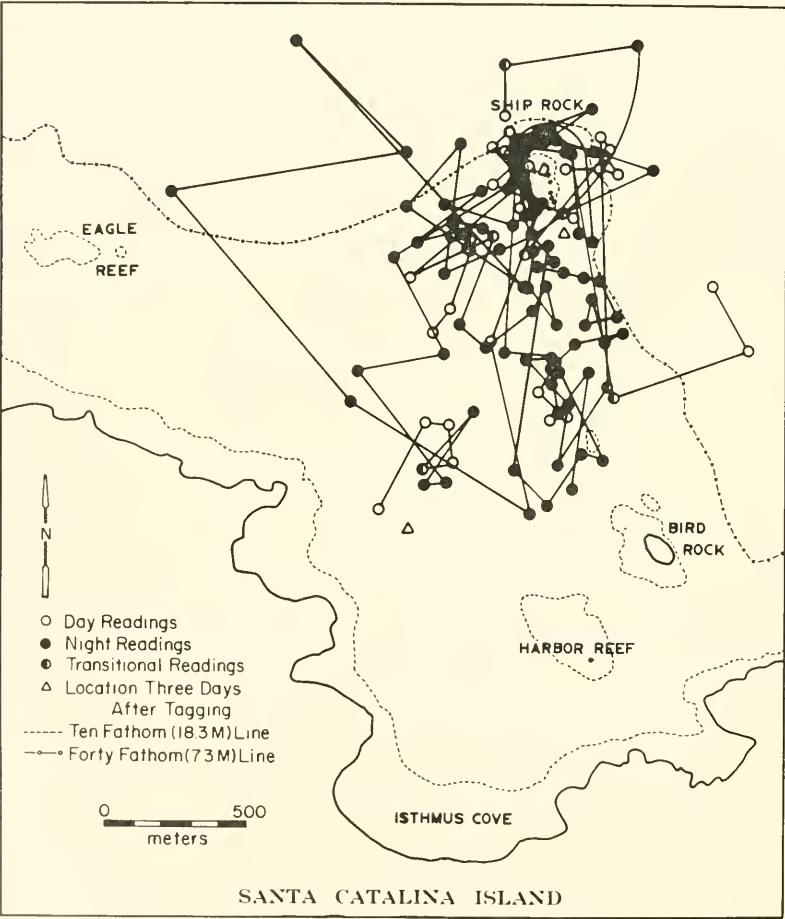
Figure 26 Positions of nine angel sharks, each tracked by E. Standora for approximately one day-night cycle. The two individuals retaining the transmitters beyond the first day (no release mechanism) were relocated 3 days later at the positions indicated, still within the general home area. All transmitter applications were at Ship Rock. (From Standora and Nelson 1977.)

Depth can be determined in addition to location if the transmitter is equipped with a pressure sensor. Biologically, depth is a very useful parameter to monitor, because sharks often show distinct daily rhythms of depth preference or of rate of change of depth. An example of continuous data covering several days from a gray reef shark equipped with a single-channel depth transmitter is shown in Figure 27.

Data from a single-channel, swim-speed transmitter can establish a shark's basic day-night activity pattern (diurnal, crepuscular, nocturnal) in terms of swimming speed and whether it ever stops swimming to rest on the bottom. The most complete picture of general activity, of course, comes from multi-channel transmitters having several different sensors.

Long-Term Movement, Migration

Since manpower requirements limit the durations of continuous ultrasonic trackings, it would appear that radio methods are most applicable for studying the longer term movements of non-home-ranging/pelagic species such as the blue shark. For instance, it would be interesting to monitor the progress of blue sharks off the California coast as they migrate northward in the spring and southward in the fall. Only the general existence of this migration is established; very few of the details are known. For example, do individuals move along the coast at relatively uniform rates or do they move more erratically, lingering at certain "temporary home ranges," such as when feeding on spawning squid at Santa Catalina Island?

Much of what is known about long-term migrations of sharks comes from standard tagging and recovery programs, but such efforts are hampered by
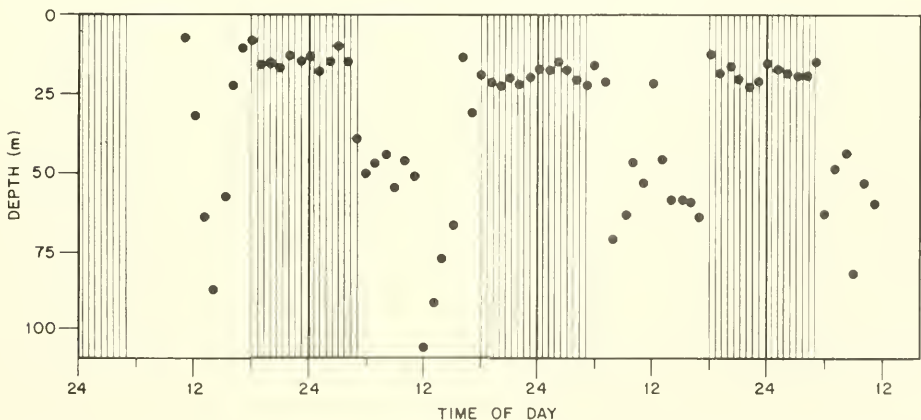
Figure 27   Example of data from a single-channel depth transmitter from one gray reef shark tracked continuously for 72 h at Rangiroa, French Polynesia. Shaded areas indicate times from sunset till sunrise. Note the distinct correlation between depth and time of day. First point is at the site of transmitter application (self-ingested in bait) in shallow water to which the shark was bait attracted. (From Johnson and Nelson, unpublished data.)

the usually very low recovery percentages. Also, the very variable and un-predictable time lapse between tagging and recovery further reduces the value of what little data are collected. A telemetering study using single-radio-release units can be likened to a conventional tagging study in which nearly 100% recovery occurs and in which recovery times can be precisely chosen in advance by the tagger. Thus, blue sharks off Santa Catalina could be tagged with single-radio units set to detach in 24 h (or 48 h, etc.), and popup locations would be determined by receiving stations on island mountaintops. Boats would then be directed to the site for transmitter recovery, unless the location were so distant that cost of the recovery mission would exceed the value of the transmitter. Longer term trackings (1 or more weeks) would probably require searching by light airplane and, furthermore, would be best accomplished by multiple-radio units. A package of at least three radios would be feasible for blue sharks; even more could be carried by larger species such as the great white shark.

An exciting future possibility, should governmental support become avail-able, is worldwide tracking from polar-orbiting satellites. An ambitious project of this type was proposed by Goodman et al. (1973) for the satellite tracking of migrating whales over periods of 1 year or more. The general route of the animal during the year would be monitored by satellite-fixing of periodically released expendable radio buoys (401 MHz). At the end of the tracking a larger multichannel data-recording package would detach for recovery by a waiting shipboard crew.

Detection of Specific Behaviors

Besides the telemetering of relatively general, long-term trends of movement, activity, or depth, the researcher may also wish to identify the occurrence of other more specific behaviors. Such "specific behavioral events" may occur only briefly and infrequently and thus may be missed by the tracker unless the sensor data are continuously recorded, either at the receiver end or in a transmitter storage unit to be later interrogated.

Feeding Behavior—Very little is known about how most large, active sharks normally obtain their food. While some data exist on what they eat (from gut contents, preferred baits), surprisingly little is known about when they feed, how often they feed, and exactly how they capture their normal prey. Telemetering techniques can be used to provide this kind of information.

One method would involve stomach-implanted transmitters that directly detected the intake of food by sensing changes in pH or temperature when food and accompanying water were swallowed, or when stomach secretions increased. Another way would be by recognition of specific events such as turns, accelerations, or jaw movements, which are probably correlated with feeding actions. Multichannel telemetry increases the probability of iden-tifying significant specific events because it allows close time comparisions between two or more factors. For example, "sudden direction change" (compass sensor) occurring during the same brief time period as "sudden

speed increase" (speed sensor) might turn out to be an event representing a shark chasing a certain type of prey. A different event might consist of "tight circling, slow speed" at a different depth, and this might indicate feeding on some other prey. Specific events of these types, even though they may occur only briefly and infrequently, would be readily apparent on computer printouts of continuously recorded data.

Courtship and Mating—While little is known about natural feeding behavior in sharks, even less is known about social behaviors such as courtship and mating. Courtship in sharks has never been described and even copulation itself has been illustrated in the literature only from aquarium observations of two small, bottom-dwelling species. For the large active species, almost nothing is known of mating, except for what can be indirectly inferred from evidences such as mating scars (tooth marks) on females or sperm in the claspers of males. Questions as to where, when, and how often mating occurs in the natural environment remain completely unanswered. It is intriguing to consider the possibility of approaching these questions with telemetry.

One way would be to search for some specific event believed associated with mating, such as flexing of the claspers. At the beginning of the suspected season of mating, mature males would be captured and fitted with long-term memory transmitters with sensors to detect clasper flexion. Data storage (as opposed to continuous tracking) would seem the most feasible initial technique, since the researchers would have very little idea of when or how often to expect the clasper events. The various units would be relocated and interrogated at intervals, e.g., daily, perhaps only weekly. When clasper activity was indicated, a shorter term memory might be used to determine more accurately the time and place of the behavior. It would then be feasible to begin continuous trackings to obtain the most detailed telemetry account of the behavior. If conditions warranted, attempts at direct observation of the behavior might be made then by receiver-equipped personnel using scuba or submersibles.

Physiological Monitoring

A good example of the use of acoustic telemetry for monitoring physiological responses in free-ranging marine animals is the work of Carey and Lawson (1973) on thermoregulation in large tunas and sharks. Here the advantage of telemetry was that it allowed the use of the ocean as a "laboratory," making it possible to work on large, fast-swimming species impossible to maintain in existing experimental aquariums. Furthermore, the ocean had the thermal structure necessary for the experiment, which involved determining whether the animals actively regulated body temperature when moving through gradients of water temperature. The experimenters found that, if necessary, they could induce the animal to dive into colder waters by running the tracking boat directly overhead, as was done with one telemetered dusky shark.

Facilitating Observation and Experimentation

Although designed primarily as a tool which gathers the desired data, teleme-
try can also be used simply to indicate the whereabouts of a transmitter-
equipped individual so that other kinds of observations or experiments can
be performed. For example, studies of the cave-dwelling habits of reef
whitetip sharks at Rangiroa, French Polynesia, were aided by marking indi-
viduals with stomach-implanted transmitters at identifiable frequencies
(Nelson and Johnson, in press). Divers using underwater receivers could
pinpoint a shark's location in a particular cave, so that its relation to other
sharks in that cave could be observed. Likewise, the practicality of divers
observing packs of adult gray reef sharks at Rangiroa was enhanced by
ultrasonically tagging one of the pack members (Johnson and Nelson,
unpublished data).

Ethological Experiments—Some accounts suggest that certain sharks
may possess geographical "territories"[9] (McNair 1975). If this is true, it
bears directly on the shark attack problem, especially for those types of
attacks now being attributed to agonistic motivations (Baldridge and
Williams 1969, Johnson and Nelson 1973). In at least one dangerous
species, the gray reef shark, agonistic motivations are often manifested
during diver—shark encounters as distinct threat displays. What is needed
to confirm territoriality would be proof that a given individual shark be-
haves more aggressively toward intruders in one part of its home range than
in other parts. Marking a shark with a UST not only would permit delinea-
tion of the shark's home range but also would facilitate experimental diver—
shark encounters at various places in the home range.

Sensory Mechanisms—Field experiments to determine sensory capaci-
ties and orientation mechanisms can also be facilitated by telemetering tech-
niques. Detailed "pictures" of a shark's swimming patterns (from compass
sensor, transponder, etc.) when detecting a test stimulus and orienting itself
to it can help elucidate the mechanism involved, e.g., whether a true direc-
tional response or gradient seeking.

For example, the finding of Sciarrotta (1974) that blue sharks make an
evening twilight migration toward the Santa Catalina Island shoreline raises
the question as to how the sharks accomplish this oriented movement
(Figure 2). Possible navigation mechanisms include sun compass, magnetic
compass, and acoustic, i.e., to sounds emanating from the island. It is hoped
that further telemetry work may elucidate the mechanism involved. One
planned experiment involves capturing sharks at one site north of the island,
then releasing half of them there, and releasing the other half at a site south
of the island (Figure 28). If either the sun or the earth's magnetic field is
providing the orientation reference, then the "south" group should move in
the same compass direction as the "north" group, thereby moving away
from the island. If the sounds of the island are used, then both groups

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[9]In the broad sense, including areas of elevated dominance.
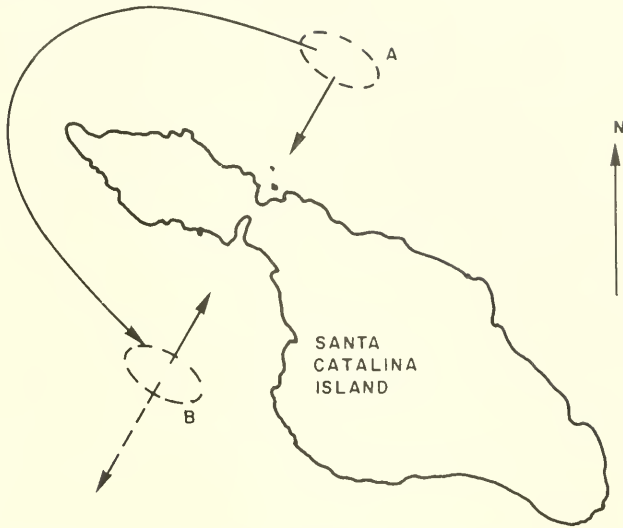
Figure 28 Proposed telemetry experiment at Santa Catalina Island, Calif., to determine the mechanism of orientation in shoreward-migrating blue sharks: (A) Capture/release site north of island. (B) Release site south of island. See text for details of experiment.

should move towards the island. If it becomes necessary to separate sun-compass from magnetic-compass mechanisms, additional experiments could be done involving eye occluders, attached magnets, etc. The trackings for these experiments need last only from about midafternoon (tagging time) until midevening. Although ultrasonic trackings would provide the fullest detail on paths taken by the sharks, two-point radio trackings would still answer the basic question of which way the animals moved, and more such trackings could be accomplished per unit time.

Another example of the use of telemetry for sensory experiments is the study proposed by A. Schuijf and the author to elucidate the mechanism of far-field directional hearing in sharks. Recent experimental evidence of Schuijf (1975) and Schuijf and Buwulda (1975) indicates the need for the gas bladder to resolve the 180° ambiguity in the directional hearing of certain teleosts. Since sharks, lacking a gas bladder, are able to "home in" accurately on low-frequency sounds from distances of at least several hundred meters (Nelson 1967, Nelson and Johnson 1972, Myrberg et al. 1972), the question arises of how they handle the 180°-ambiguity problem. A critical aspect of the problem concerns the behavior of the shark at the moment it first responds to the sound. For instance, does it always turn in the correct direction or does it sometimes turn in the opposite direction but eventually double back onto the correct direction? Does it respond immediately, turning sharply to the correct course, or does it circle slowly for a while before selecting a direction? Since the responding sharks are normally too distant to be seen by underwater observers, it is planned to

use telemetry to provide the necessary "picture" of the shark's path imme-diately after reception of the low-frequency attractive sounds. In acous-tically suitable home-range areas, it is planned to establish several indi-viduals carrying transponder or timefix transmitters, possibly also with compass sensors. The experimental low-frequency playbacks can then be made on any subsequent day, whenever a telemetered shark passes by at the desired distance and direction.

ACKNOWLEDGMENTS

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V ELECTRICAL SENSES

PHYSIOLOGY OF THE AMPULLA OF LORENZINI, THE ELECTRORECEPTOR OF ELASMOBRANCHS

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INTRODUCTION

Ampullae of Lorenzini are found in all elasmobranchs and presumably are
homologous within the group. Although these receptors are sensitive to
thermal, mechanical, and even salinity changes, behavioral experiments
(described elsewhere in this volume by Kalmijn (1978); see also Murray
(1974)) make it clear that they are electroreceptors. Moreover, they are
extremely sensitive, responding to stimuli a few microvolts in amplitude.
Among electroreceptors ampullae of Lorenzini are classed as tonic, low-
frequency or ampullary receptors. They are called tonic and low-frequency
because there is a steady resting discharge in the afferent fibers and they
respond quasi-tonically to quite low frequency stimuli. They are called
ampullary because the receptor epithelium is an ampulla connected to the
exterior by a canal of variable length (Figure 1).

Figure 1 Ampulla of Lorenzini
(after Waltman 1966). The ampulla
consists of a cluster of alveoli, one
of which is shown in cross section.
The receptor cells are innervated
by about five afferent nerve fibers
that ramify profusely over the sur-
face of the alveoli. The neck of the
ampulla, where the receptor cells
are no longer found, is called the
marginal zone (MZ).
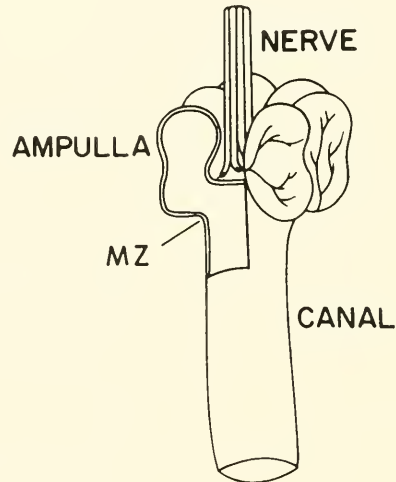
The ampulla contains the sensory and supporting cells of the sensory
epithelium; the jelly-filled canal provides a good electrical connection
between the ampullary lumen and the exterior. For purposes of physio-
logical analysis a few points may be noted here: (1) Between the receptor
cells, supporting cells, and cells of the wall of the ampullary canal are
extensive tight or occluding junctions (*zonulae occludentes*) that restrict the
flow of electric current through intercellular clefts (Figure 2); transepithelial
current passes through the cells rather than between them. (2) The receptor
cells have only a small portion of their total surface distal to the *zonulae
occludentes* and facing the lumen; by far the greatest part of their
membranes is basal face. (3) In the basal faces are the afferent synapses,
which have large presynaptic ribbons with vesicles lined up on their surfaces;
the morphology is typical of acoustico-lateralis receptors known to transmit
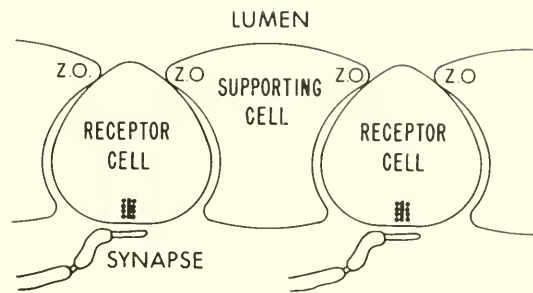
Figure 2 Diagram of cell types of the sensory epithelium. Two receptor cells are adjoined by supporting cells. *Zonulae occludentes* (ZO) partition the cell membranes into lumenal and basal faces. In the basal region are characteristic ribbon synapses with the afferent fibers. (From Clusin and Bennett 1977*a*.)

chemically. About five afferent fibers innervate each ampulla. Efferent synapses are absent.

There is some historical merit and heuristic simplicity in describing first the inferred operation of teleost tonic receptors; the comparison will be made later in this exposition in any case. The morphology of teleost tonic receptors is essentially the same, although the lumenal face is a somewhat greater fraction of the total membrane area. Stimuli applied across the epithelium are developed largely across the basal face because the lumenal face is inexcitable and of relatively low resistance (Bennett 1971*b*, *c*). External positivity causes outward current through the basal face, tending to depolarize it. There is in the absence of stimulation a resting Ca activation and influx that mediate the tonic release of transmitter that causes the tonic nerve activity. Further depolarization causes increased Ca activation and increased release of transmitter; hyperpolarization decreases Ca activation and reduces transmitter release. In freshwater teleosts these changes in Ca conductance themselves cause little change in membrane potential, and in terms of stimulus strength versus impulse frequency the receptor behaves quite linearly over a moderate range of stimuli; stronger stimuli cause a flattening of the impulse frequency relation or block nerve activity entirely.

Although Ca activation caused by depolarization leads to inward current and a regenerative response, evidently the evoked currents are too small to cause significant nonlinearities, at least over a moderate range. An analogous situation is observed in the squid giant synapse, in which a Ca action potential can be demonstrated only when K activation is blocked and the driving force for Ca is increased by increasing external Ca (Katz and Miledi 1969). In the marine catfish *Plotosus* a regenerative Ca response can be recorded (Akutsu and Obara 1974, Obara 1974, 1976).

The mode of operation of the ampulla of Lorenzini is distinctively different from that of teleost receptors (Obara and Bennett 1968, 1972). A

lumen-positive stimulus is inhibitory in terms of nerve discharge, and a lumen-negative stimulus is excitatory. This polarity inversion results from excitability of the lumenal faces of the receptor cells. A lumen-negative stimulus depolarizes and excites the lumenal faces. Their regenerative depolarizing response depolarizes the basal faces so that they secrete transmitter. There is activity of the receptor cells even in the absence of applied stimuli. A lumen-positive stimulus decreases this activity and leads to relative hyperpolarization of the basal faces and reduced release of transmitter. The detailed evidence for these mechanisms is described in the next sections.

Excitability of the Lumenal Membranes of the Receptor Cells

One of the beauties of the ampulla of Lorenzini as an experimental preparation is that it can be removed from the animal with sections of canal and nerve attached. The canal can then be rinsed with nonelectrolyte (sucrose-urea) solutions to electrically isolate the epithelium. The responses to controlled currents or voltages applied across the epithelium in such a preparation can be recorded from the distal end of the canal or by electrodes penetrating it near the ampulla (Figure 3).

The nerve response also can be recorded. In the presence of tetrodotoxin (TTX), nerve impulses are blocked and postsynaptic activity ("postsynaptic potentials," or PSPs) that initiate impulses can be measured by external electrodes with relatively little distortion. A further important advantage is that the canal and basal surface of the epithelium can be independently perfused with experimental solutions.

When the ampulla is electrically isolated it develops a lumen-positive resting potential of about 10–30 mV (Clusin and Bennett 1977a). Moderate lumen-positive rectangular current pulses and small lumen-negative ones produce exponentially rising and falling voltages (Figure 4A, B) and give a linear voltage current relation (Figure 5). The receptor epithelium behaves like a simple resistance and capacity in parallel. Somewhat larger lumen-negative stimuli, at a threshold of about 0 mV, elicit a regenerative response, an all-or-none action potential up to 100 mV in amplitude and about 100 ms in duration (Figure 4A, C). This action potential is lumen negative and is an active response because it can outlast and is much larger than a near-threshold stimulus (Figure 4C). Its polarity is consistent with its being a depolarizing response of the lumenal faces, and we have generally displayed it lumen-negative-upward so that it appears like an ordinary action potential recorded intracellularly from a single cell. Actually, the ampulla behaves in many respects like a single, inside-out cell.

The origin of the response as a calcium spike in the lumenal membranes is established by perfusion experiments (Clusin and Bennett 1977a). Drastic changes of ions bathing the basal face have little or no effect on the response. Perfusion of the lumen with Co, an ion that blocks Ca channels in many tissues, blocks the response as does zero Ca, EGTA solution. TTX,
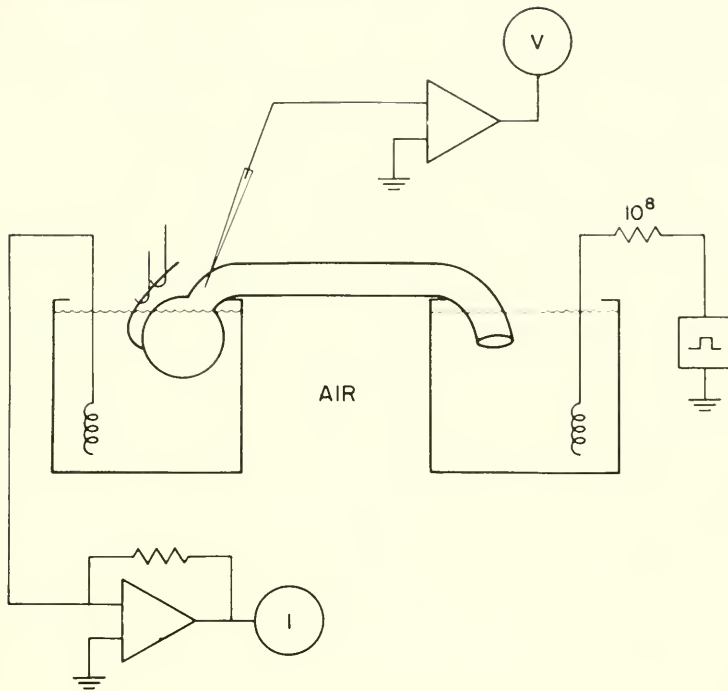
Figure 3   Experimental setup for passing constant current across the epithelium and recording its responses. The isolated ampulla lies in one pool, and its canal crosses an air gap to a second pool. Current applied between pools enters the canal and exits mainly through the ampullary epithelium. A microelectrode in the lumen records resulting transepithelial voltages, and current is measured by an operational amplifier. The nerve is led into an oil-filled pipette, and its responses are recorded by fine wires. (From Clusin and Bennett 1977a.)

which blocks Na channels in many cells, has no effect when applied to either face.

Voltage clamp experiments allow further characterization of the response of the lumenal membranes. For lumen-positive clamping pulses, the epithelium behaves linearly. For lumen-negative stimuli, there is an inward-outward current sequence like that observed in excitable cells in general (Figure 5A, C). The inward current leads to the regenerative rising phase of the action potential, and the outward current terminates the response. There are, however, several important differences between these records and those from, for example, the squid axon. If one plots the peak inward current vs voltage, the relation is linear from about 25-mV lumen-negative to very large lumen-negative stimuli. When the late current is plotted, this relation is also linear over an appreciable range, but for large lumen-negative stimuli the onset of the outward current is progressively delayed (Figure 5A, 71–119 mV) and finally blocked altogether (148 mV).
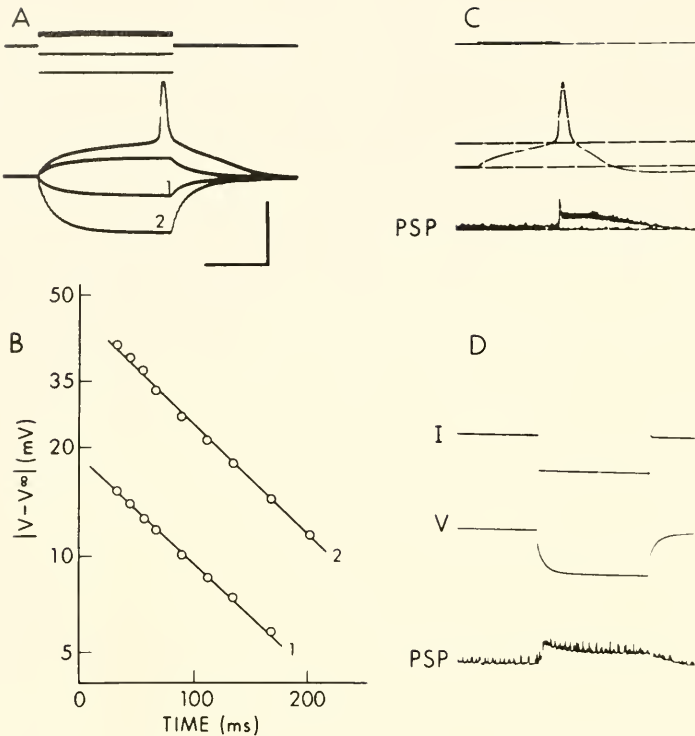
Figure 4   Effects of constant current on the ampullary epithe-
lium. Applied current is shown in the top trace, lumen-negative
stimuli upward. The transepithelial voltage appears below with
the lumen-negative responses shown upward. In A, hyperpolar-
izing and subthreshold depolarizing stimuli cause the epithelium
to exponentially approach a voltage that is proportional to ap-
plied current. There is a 30-mV lumen-positive resting potential,
the threshold for the action potential being about 0 mV. In B, a
semilog plot of voltage minus final voltage vs time after stimulus
onset confirms the exponential time course of the passive re-
sponse. The time constant of the inactive epithelium is 126 ms
(average value from plots 1 and 2 in B). The inactive or leakage
resistance of the epithelium is 372 kΩ and the capacity is there-
fore 0.34 μF. An ampullary action potential and corresponding
postsynaptic potential (PSP) are shown in C. The trace passing
through the base of the action potential parallel to the base line
indicates 0 mV. The extracellular recording of the PSP (bottom
trace) shows superimposed action potentials arising in the nerve
fibers. There is no change in postsynaptic activity from the
spontaneous level until the threshold is reached. In D, transmitter
is released by a strong lumen-positive stimulus which directly
depolarizes the basal, secretory membranes of the receptor cells.
The current calibration (vertical bar) is 0.4 μA in A, 1.0 μA in C,
and 2.0 μA in D. The transepithelial voltage calibration (vertical
bar) is 70 mV in A, 40 mV in C, and 200 mV in D. The post-
synaptic voltage calibration (vertical bar) is 2.0 mV in C and
0.8 mV in D. The time calibration (horizontal bar) is 0.4 s in A
and C and 0.2 in D. From Clusin and Bennett 1977a.
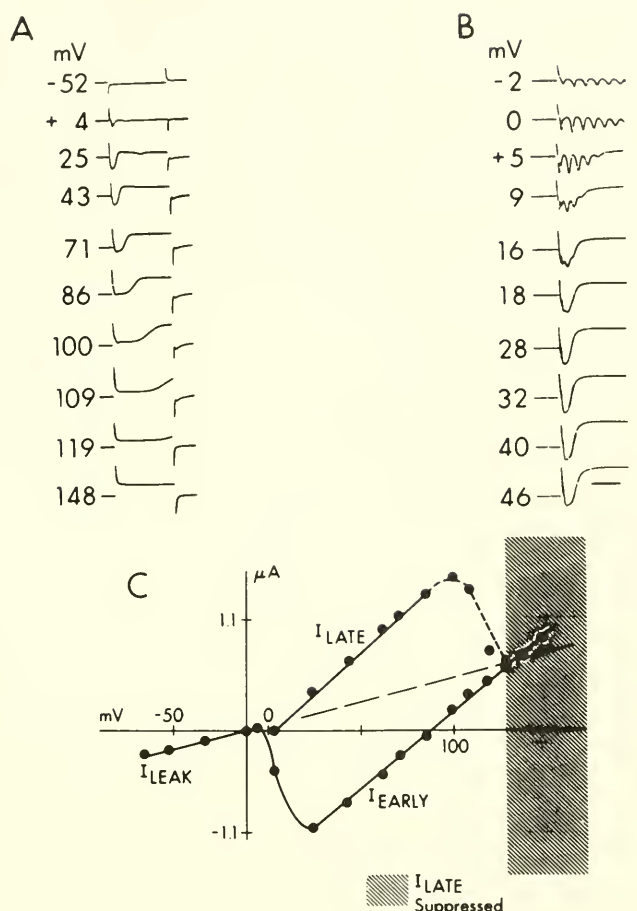
Figure 5   Active currents of skate electroreceptor epithelium. Figure 5A shows a family of currents obtained when constant voltage displacements were imposed across an ampulla of standard size. Current flowing inward across the lumenal membranes is defined as inward current and shown downward. Voltage displacements that depolarized the lumenal membranes (lumen negative) are defined as positive. The epithelium was held at its resting potential of -12 mV. Lumen-positive stimuli evoked maintained leakage currents. Lumen-negative stimuli between 0 and +88 mV evoked an early inward current followed by a late outward current. With larger excitatory stimuli, onset of the late outward current was progressively slowed and delayed. At +128 mV, activation of the late outward current was suppressed. The currents obtained at +4 and +25 mV shows a secondary peak of inward current. A current vs voltage relation of the data in A is plotted in C. The vertical axis is placed at the holding potential. The leakage current (I_{LEAK}), the peak early current (I_{EARLY}), and the late outward current (I_{LATE}) are linearly related to voltage over broad ranges. The slope resistance during the early current is 64 kΩ within the linear range, while that of the late outward current is 58 kΩ. The leakage resistance is 203 kΩ. The current traces in B are from an electroreceptor whose current—voltage relation (not shown) is similar to that in C. However, the traces in B show several inward current peaks during small stimuli. These inward current peaks attenuated with progressively larger stimuli. Above 28 mV, only one peak occurred. The holding potential in B was -18 mV. The vertical calibration represents 1 μA in B and 2.4 μA in A. The horizontal calibration represents 100 ms in B and 220 ms in A. (From Clusin and Bennett 1977b.)

Extrapolation of the resting resistance line and the peak inward current line shows that the point at which the Ca current reverses is the point at which the late current fails. We have termed this point the suppression potential for the late outward current. It is analogous to the suppression potential at the squid giant synapse, which is the potential, inside positive, at which transmitter release is blocked (Katz and Miledi 1967, Kusano et al. 1967). It is also the point at which Ca entry fails (Llinás and Nicholson 1975), and thus it is the same as the Ca equilibrium potential. If the lumen of the ampulla is perfused with low Ca solution, the potential required to reverse the early current is reduced, but it remains identical to the suppression potential for the outward current (Clusin and Bennett 1977b). If Ca entry is blocked by Co or zero Ca, there is no late outward current. The conclusion we have reached is that this outward current is activated by calcium influx. Comparative considerations suggest that it is a K current, although we cannot yet exclude a Cl contribution. Calcium-activated K conductances have now been found in a number of excitable as well as inexcitable cells (cf. Meech and Standen 1975).

When activation of the late current is blocked, the early current persists unabated; it shows little or no inactivation. This is a property commonly found in Ca conductance systems (Katz and Miledi 1969).

When the epithelium is repolarized after a clamping pulse that activates the late outward current, there is a tail current that lasts about 600 ms (Clusin and Bennett 1977b). When only Ca activation has occurred tail currents are much briefer and are difficult to distinguish from the capacitative transients. Thus Ca activation reverses relatively quickly, while activation mediating the late outward current is much longer lasting. Presumably its duration represents, in part, time for reduction in cytoplasmic Ca concentration by sequestration or extrusion.

The responsiveness of the ampulla must arise, at least in part, in the receptor cells, because PSPs and afferent discharges are associated with the action potential generated by the electrically isolated epithelium (Figure 4C). From measurements of the electrical capacity and membrane areas of the ampulla, we have inferred that the supporting cells behave as passive elements during the ampullary response.

Equivalent Circuit of the Epithelium

In the preceding discussion the ampulla was seen to behave like a single inside-out cell. In this section we analyze the complexities due to the series resistance of the basal membranes of the receptor cells and the shunt pathways around these cells. An equivalent circuit of the epithelium, in which all the receptor cells are lumped together, is given in Figure 6. There are three resistances in the lumenal membrane of the receptor cells: the resting or leakage resistance, that of the voltage-sensitive Ca system, and that of the Ca-activated outward current system. There are also the resistances of the basal membranes and the shunt pathway. This is a total of five
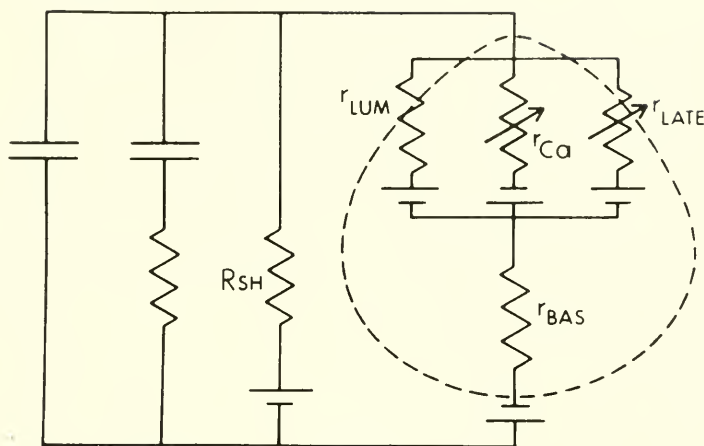
Figure 6   Equivalent circuit of the ampullary epithelium. Active currents arise in the lumenal membranes of the receptor cells. These lumenal membranes are represented by a fixed resistor r_{LUM} in parallel with two variable resistors. The early calcium current flows through r_{Ca} and the late current through r_{LATE}. The basal faces of the receptor cells are represented by fixed resistance r_{BAS}. Most of the leakage current flows through the supporting cells, the marginal zone cells, or the intercellular clefts. These pathways are represented by a single resistor R_{SH}. The batteries E_{Ca}, E_{LUM}, E_{LATE}, E_{BAS}, and E_{SH} are drawn but not labeled. The ampullary capacity is attributed to cells that are electrically in parallel with the receptor cells, namely the supporting cells and marginal zone cells, whose basal membrane area is large. Some of the ampullary capacitance is in series with a significant fixed resistance. (From Clusin and Bennett 1977a.)

resistances (for d.c. measurements the resistance in series with one of the capacities is irrelevant).

The voltage current relation of the epithelium allows measurement of only three slopes. However, further information is available. As an excitatory stimulus is increased, current flowing inward through the lumenal membranes and outward through the basal membranes is reduced. Consequently, the basal membranes are less depolarized and less transmitter is released. If shunt resistance R_{SH} were infinite, there would be no current inward through the receptor cells, when there was no net current across the epithelium. However, transmitter release persists well beyond the point at which net epithelial current changes in sign. Evidently in this range current flows inward through the lumenal membranes and on outward through the basal membranes, while a larger current flows outward through the shunt resistance.

If one assumes the basal membranes to be sensitive to outward current, as they should be in an electroreceptor, the point at which the PSP fails will be close to the point at which all the current across the epithelium is going

through the shunt resistance. This allows a simple and quite direct measurement of the shunt resistance. It turns out that the shunt resistance is low compared to the series sum of lumenal leakage and basal membrane resistances of the receptor cells; at least 90% of the current flows across the shunt resistance. (In the short-circuited ampulla the receptor cells are active and more of an applied current flows through them, as described later.)

The voltage—current relations show that the resistance of the epithelium decreases when the Ca current is activated and decreases further when the late outward current turns on. Because of the shunt resistance the change in the receptor cell resistance is much greater than the change in the overall epithelial resistance. The decrease is about twentyfold for Ca alone and thirtyfold for both conductances. If all the residual resistance is in the basal faces of the receptor cells, then the resting resistance of the lumenal membranes must be 30 times as great as the resistance of the basal membranes. The importance of this observation is that most of a voltage applied across the inactive epithelium is developed across the lumenal membranes. It also allows one to obtain a good approximation of the resting resistance of the lumenal membrane from total resting and shunt resistances.

There remain three uncharacterized resistances and two slopes in the voltage—current relation. The third independent variable required for solution of the circuit comes from measurement of three potentials: the Ca equilibrium potential, the reversal potential for when both Ca and late current are activated together, and the reversal potential of the late current alone after repolarization of the epithelium when the Ca conductance has returned to normal but the late conductance persists. From these three numbers, neglecting r_{LUM} and r_{BAS} (see Figure 6), one obtains the ratio of the Ca and late resistances, which with the slopes during Ca and late currents allows calculation of the other values.

The details of the calculation are given in Clusin and Bennett (1977b) and will not be repeated here. The important result is that the basal membrane resistance, while small compared to the resting lumenal membrane resistance, is considerably larger than the resistance of the active membrane (Table 1). Thus, in their negative slope regions the membrane potentials cannot be clamped. This property appears essential to the operation of the ampulla as an electroreceptor (see below) but prevents accurate measurement of the kinetics of the Ca and late conductances.

The potentials across the cell membranes are important quantities that cannot be measured by transepithelial measurements. Equilibrium potentials are measured only as changes from the zero or resting potential across the epithelium. As noted, the isolated epithelium shows a lumen positive resting potential of 10 to 30 mV. This value is largely determined by the potential of the shunt pathway most of which would appear to be a relative hyperpolarization of lumenal as compared to basal membranes of the supporting cells. If these membranes are similarly selective to the membranes of the receptor cells, the transepithelial potential would represent the true difference in resting potential in both kinds of cells and no currents would flow through the receptor cells and shunt pathway in the isolated ampulla.

Responsiveness of the Basal Faces

One would expect that the basal faces had some excitable Ca channels because transmitter release is produced by depolarization of this face, requires Ca, and is blocked by high Mg solutions (Steinbach 1964). Excitability of the basal faces can be demonstrated in ways similar to those used with other gradedly responsive systems, such as the presynaptic fiber of the squid giant synapse (Katz and Miledi 1969). If 2-mM tetraethyl-ammonium (TEA) and high Ca are applied to the basal membranes or if Ba is substituted for Ca, these membranes become able to generate a prolonged action potential (Figure 7). This response is recorded as a long-lasting, lumen-positive potential. It can be evoked by lumen-negative stimuli that excite the lumenal membranes, which then excite the basal membranes; it then follows the briefer lumen-negative action potential of the lumenal membrane (Figure 7C). Alternatively, it can be evoked by lumen-positive stimuli that directly depolarize the basal membranes (Figure 7D). The long-lasting responses of the basal faces are associated with large and long-lasting PSPs in the afferent nerve. The excitability of the basal faces in normal solutions has little effect on the voltage—current relations of the epithelium described in the previous section, but it is important in electroreception, as discussed in the next section.

Electroreception

When the ampulla and canal preparation is held short circuited to zero transepithelial potential (by a salt bridge connecting the two pools of Figure 3), the receptor epithelium is in its negative slope region. Because of the low

Table 1. Membrane resistance values

	Whole ampulla, r/n	One receptor cell, r	1 cm^2 of membrane, $r \times$ area
	kΩ	mΩ	Ω cm^2
r_{LUM}	$\geqslant$3,800	$\geqslant$38,000	$\geqslant$3,000
r_{BAS}	112 $\pm$ 6	1,120 $\pm$ 60	5,500 $\pm$ 300
r_{Ca}	48 $\pm$ 6	480 $\pm$ 60	38 $\pm$ 5
r_{LATE}	6.1 $\pm$ 3.0	61 $\pm$ 30	4.9 $\pm$ 2.4
$1/(G_{Ca} + G_{LATE})$	5.4 $\pm$ 2.5	54 $\pm$ 25	4.3 $\pm$ 2.0

The values of r_{Ca} and r_{BAS} are based on the assumption that E^*_{LATE} = − 7 mV and R_{SH} = 352 kΩ. The errors represent maximum deviations of the listed value from values calculated with the extreme assumptions E^*_{LATE} = 0 and − 12 mV, and R_{SH} = 322 and 352 kΩ. The calculated value of r_{LUM} does not depend on E^*_{LATE}. Values for a single cell (second column) assume 10^4 cells. Values in the third column assume the receptor cells to have a lumenal membrane area of 8 μm^2 and a basal membrane area of 500 μm^2. From Clusin and Bennett (1977b).
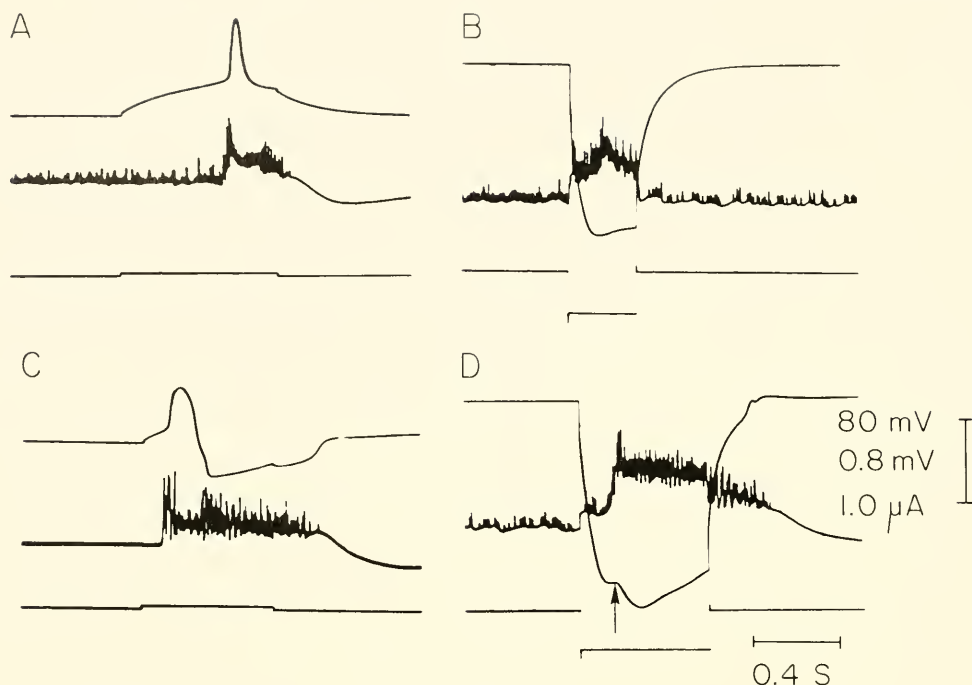
Figure 7   Evidence for excitability of the basal faces of the receptor cells recorded as in Figure 9, but at lower gains. (A) Normal monophasic action potential recorded across the current-clamped ampulla in physiological saline. (B) Strong lumen-positive stimuli can cause nerve responses by direct depolarization of the basal faces. Inexcitability of the presynaptic membrane in physiological saline is indicated by the absence of a positive-going regenerative response and by the immediate cessation of transmitter release at the end of the stimulus. The transepithelial potential declines slightly during the response. This presumably results from membrane breakdown associated with the large stimuli applied, although slight responsiveness of the basal faces cannot be excluded. There is some direct pickup in the nerve recording of this very large stimulus. (C, D) The basal faces become excitable when perfused with 20-mM calcium and 2-mM TEA: (C) Stimulation of the lumenal membranes by a lumen-negative stimulus produces a diphasic receptor action potential, the prolonged lumen-positive phase arising in the basal faces. This response is associated with a large PSP and intense postsynaptic impulse activity. (D) Direct stimulation of the basal faces by a lumen-positive stimulus produces a lumen-positive regenerative response (onset at arrow), which starts during the stimulus and ends after its termination. This response is also accompanied by augmented transmitter release and postsynaptic impulse activity.

resistance of the canal, the epithelium is rather well clamped at zero potential. However, as stated in the previous section, the receptor cells cannot be clamped at this potential because of the resistance of the basal membrane. And high gain recordings of the epithelial potential show small oscillations that wax and wane (Figure 8A, C). Evidently the individual receptor cells (of which there are several thousand) are not clamped but are oscillating under these conditions. If one applies an inhibitory
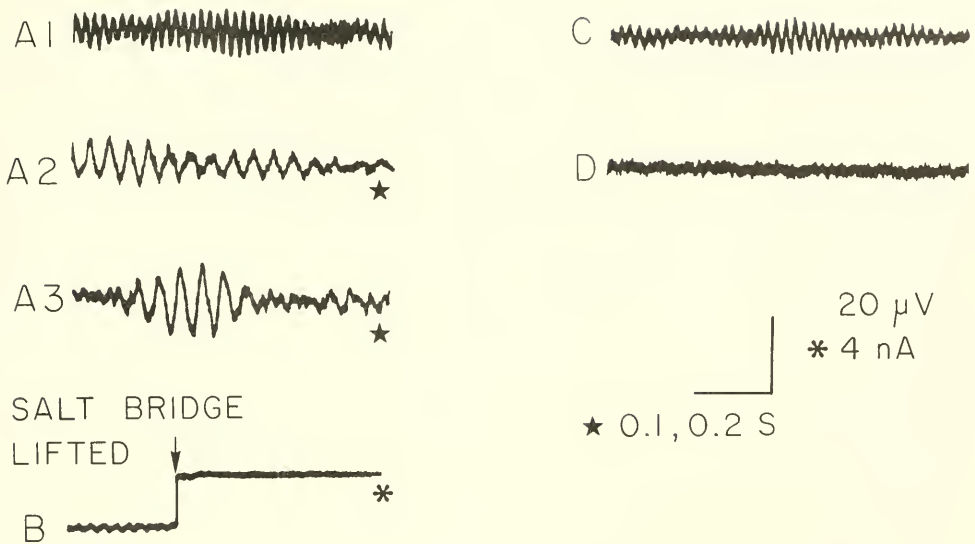
Figure 8   Spontaneous activity of the short-circuited receptor. (A1-A3) Oscillations recorded in an ampulla short circuited by a salt bridge. (B) Transepithelial current measured as voltage drop along the canal before and after lifting the salt bridge. There is a steady inward current through the lumenal membranes (displayed downward) with small superimposed oscillations until the connection provided by the bridge is removed, after which essentially zero current flows along the canal. (C, D) Oscillations recorded in the short-circuited ampulla (C) are abolished when the ampulla is made 4 mV positive (D) (high-gain a.c.-coupled recordings). (From Clusin and Bennett 1978*a*.)

(lumen-positive) stimulus of a few millivolts the oscillations cease (Figure 8d). In the short-circuited condition there is a steady inward current through the lumenal faces with small oscillations superimposed. Unshorting the ampulla blocks this current (Figure 8B).

Oscillations corresponding to the oscillations in the ampulla can be recorded from the afferent nerve. These oscillations are somewhat delayed, which indicates that they are in fact PSPs and not electrical pickup from the receptor cells (Figure 9C, D). As would be expected, the polarities are such that a response of the lumenal membranes of the receptor cells leads to excitation of the nerve. Although the steady level of postsynaptic activity is difficult to measure directly, evidently the tonic activity of the receptor cells causes tonic PSP activity in the nerve.

Excitatory stimuli of a few microvolts increase excitation of the nerve and inhibitory stimuli decrease it (the stimuli are 5 and 15 μV in Figure 9A, B). Presumably there is a change in the oscillations of the receptor cells over the population as a whole, but it is difficult to be sure from single sweeps because of the irregularity of the background oscillations. Only moderately larger stimuli produce clear changes in the amplitudes of the oscillations (Figure 9C).
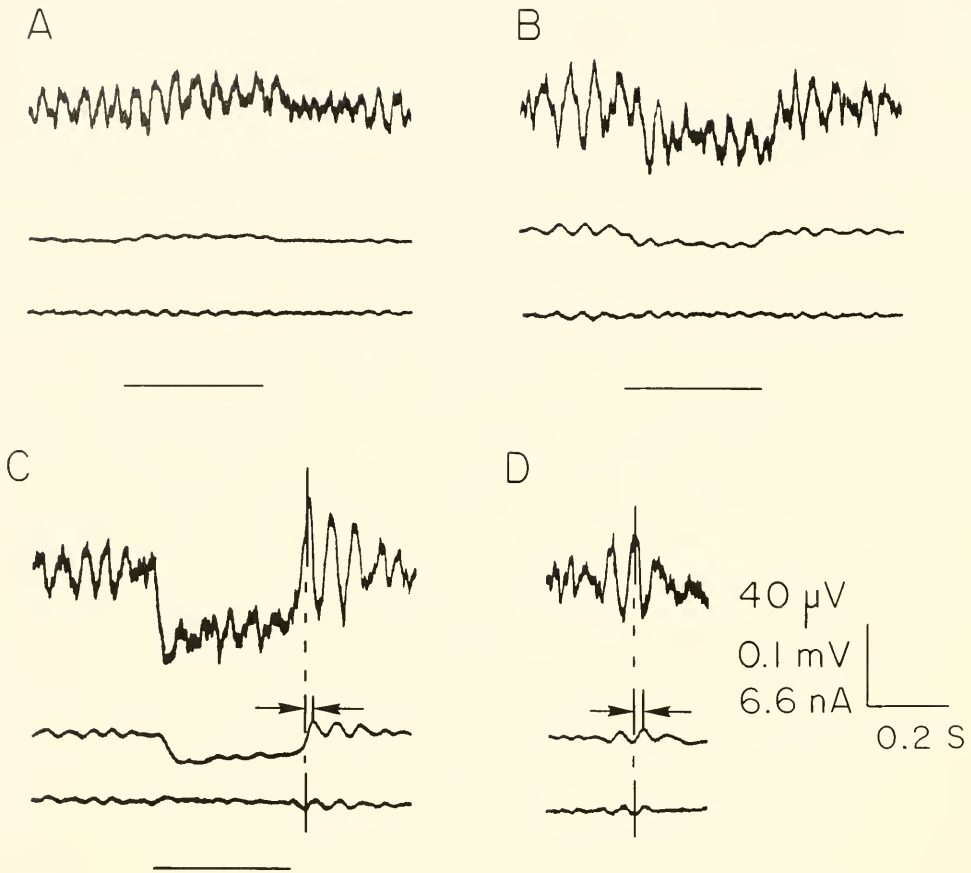
Figure 9   Responses of the short-circuited receptor to small voltage stimuli. Upper trace represents voltage recorded across the ampullary epithelium (lumen-negative up); middle trace, PSP recorded from the afferent nerve bundle (proximal electrode positive recorded up); lower trace, current across the epithelium measured as voltage drop along the canal (as in Figure 8B). (A) A lumen-negative stimulus of about 5 μV produces a clear change in the PSP in the excitatory direction, indicating increased transmitter release. (B) A lumen-positive stimulus of about 15 μV produces a change in the PSP in the inhibitory direction ascribable to decreased release of excitatory transmitter. (C) Clear oscillatory responses produced at the termination of a somewhat larger lumen-positive stimulus lead to oscillatory PSPs in the nerve with a receptor peak to nerve peak delay of about 40 ms. (D) A spontaneous oscillation and associated PSP in the nerve similarly delayed. (From Clusin and Bennett 1978a.)

Recent experiments demonstrate that responsiveness of the basal membranes plays a role in the oscillatory behavior of the epithelium (Clusin and Bennett 1978b). As noted above, the series resistance of the basal membranes prevents clamping of the potential across the lumenal membrane in its negative slope region. Changes in the resistance or effective internal potential of the basal membrane would also affect the epithelial activity. In

voltage clamp the transepithelial potential is constant; i.e., the difference between the potential of lumenal and basal membranes is the clamped potential, but within this constraint the potentials can change leading to oscillatory currents. Many voltage-clamped receptors show damped oscillations at a frequency of about 20/s when stepped into their negative slope region (Figure 5B). These oscillations are similar in time course to those in the short-circuited receptor, and presumably the mechanisms are similar in each case. The oscillations that occur in the negative slope region of the clamped receptor do not involve any outward current through the receptor cells as shown by subtraction of current through the leakage pathway. Thus, the oscillations could not involve repolarization of the lumenal membranes produced by activation of the late outward current of the lumenal membranes. However, activation of an outward current in the basal membranes cloud lead to repolarization of the lumenal membranes.

The role of the basal faces in generating the oscillations is demonstrated by perfusing these membranes with 2 mM TEA, 7 mM Co, or 5 mM [ethylene bis(oxyethylenenitrilo)]tetraacetate (EGTA). The oscillations in voltage clamp are blocked by each treatment and the epithelial response becomes greatly simplified in the negative slope region. The responses to larger stimuli are, as previously noted, essentially unaffected by these treatments, which indicates that the active conductances in the basal membranes are relatively small. Blocking by Co and EGTA could mean that there is a Ca-activated outward current in the basal faces. However, the effectiveness of 2 mM TEA in blocking the responses suggests that the basal membranes have a voltage-sensitive K conductance, for Ca-activated K conductances are generally relatively insensitive to TEA (Meech and Standen 1975). (The more rapid turnoff of the basal outward current as compared to the lumenal outward current does not imply voltage sensitivity rather than Ca activation, because it could result from more rapid removal of Ca from the cytoplasm in this region of the cell.) Blocking by Co and EGTA may mean that depolarization of the basal membranes adequate to activate a voltage-sensitive outward (K) current requires Ca activation in these membranes.

To summarize, the oscillations are probably generated as follows: Ca activation in the lumenal membranes caused by an appropriate excitatory stimulus generates an inward current that depolarizes the basal faces and allows both membrane potentials to go more positive regeneratively. Ca activation in the basal faces may contribute to this potential change. Either voltage-sensitive or Ca-activated outward current in the basal faces then reduces the potential across these faces and consequently that·across the lumenal faces as well. Ca activation in the lumenal membranes rapidly declines when they are sufficiently repolarized. With some delay the outward current-carrying conductance in the basal membranes declines; their potential and that of the lumenal membranes goes more positive and Ca activation again occurs regeneratively. And so it goes.

The oscillations generated by relatively large stimuli show rather little change in frequency with stimulus strength over a wide range of amplitudes

(Figures 9C and 5B). The damping of the oscillations may result from damping of the responses of individual receptor cells, decrease in the number of cells oscillating, and desynchronization of the responses of individual cells. Intracellular recording from single receptor cells is likely to be required for a more complete analysis and for an estimation of the magnitude of the responses.

Oscillations of 20/s in the clamped or short-circuited preparations are considerably faster than the few-per-second oscillations seen in current clamp, and their period is short compared to the duration of the late outward current. In current clamp, the canal is electrically isolated and outward current in the basal face can repolarize the lumenal face only through the shunt resistance (Figure 6). Thus the lumenal action potential apparently continues until there is activation of the long-lasting outward current in this face. The long duration of this current accounts for the relative slowness of repetitive discharges under current clamp.

The question still remains as to how the high sensitivity arises. As pointed out by Cole et al. (1970), the voltage gain of a membrane poised near threshold becomes very large in that a small applied potential produces a much larger change in potential, either generating an action potential or returning the potential to the resting level. It seems likely also that the gain of a tonically active cell can be quite large, and we ascribe the function of increasing gain to the oscillations of the ampullae. Terzuolo and Bullock (1956) found the crayfish stretch receptor to be sensitive to very small currents applied extracellularly when it was tonically active but not when it was inactive. (They were recording extracellularly, and transmembrane voltages were not measured.)

High sensitivity requires that the membrane stays poised near threshold. From the observation that many receptors are spontaneously active, it appears that adjustment to near threshold requires a tolerance of a certain amount of spontaneous activity; that is, unless a cell occasionally exceeds threshold it may not "know" that it is close to threshold. In addition, activation and inactivation during subthreshold responses may have to be reset by going through the action potential cycle. A further point about tonic activity is that it allows the receptor to respond by a decrease in activity and thus signal both increases and decreases in stimuli (or stimuli of opposite sense). The hypothesized augmentation of sensitivity associated with action potential generation requires confirmation by calculations from voltage clamp data. Relevant comparative data are considered below.

In considering the sensitivity of the receptor in other aspects than voltage-to-voltage amplification, we might suspect a more sensitive relation between voltage and transmitter release at the afferent synapses. (Since transmitter release appears on morphological and comparative grounds to be quantized, and since single quanta are detectable by many ordinary synapses, there is no reason to suppose that electroreceptor synapses have larger quanta or more sensitive transduction of transmitter concentration to postsynaptic impulse frequency.) In tonic receptors of freshwater teleosts, there is indeed an

extraordinarily sensitive relation between transmitter release and presynaptic voltage (Bennett 1971*b*, *c*). However, in the ampulla of Lorenzini the oscillations in individual receptor cells may have such large amplitudes that no unusual sensitivity of the secretory process is required.

Accommodation

A highly sensitive low-frequency receptor is analogous to a high-gain d.c. amplifier, and each needs a balance adjustment to remain in its operating range. In the skate the electroreceptor accommodates more or less completely to moderate stimuli but does not lose its incremental sensivity. That is, it is still sensitive to very small voltage changes (Murrary 1965*b*). This property is of obvious value to an electroreceptor that could be subjected to steady voltages from external sources or changes in resting potentials due to slight changes in the compositions of body fluids.

The tonic activity of the receptor cells may be an important factor in the accommodation. An increase in activity would cause an increased Ca influx through the lumenal membranes; this would increase the Ca-dependent outward current and tend to reduce the activity. If activity were lowered, cytoplasmic Ca concentration would drop, turning off Ca-activated channels and allowing the cell to depolarize. Presumably, with appropriate activation curves and rates of Ca removal these mechanisms could give the requisite uniformity of incremental sensitivity. Of course one could give the same description substituting voltage-sensitive outward current for Ca-activated outward current. However, it may be that the Ca-activated mechanism gives the cell a better "memory" of its recent activity pattern. Accommodation does occur, at least in part, in the receptor cells as demonstrated by the fall-off in transepithelial voltages during small stimuli (Figure 9C and much more extensive observations.)

A component of accommodation may be mediated by the afferent nerve, as suggested by Murray (1962). However, his observations of accommodation during polarization applied to the nerve should be reexamined in view of the more recent demonstration that the receptor is sensitive to electric stimuli that could be applied to the ampulla by the nerve itself. The nerve does not appear to accommodate to stimuli lasting for periods of the order of the interval between spontaneous impulses. If one or several extra impulses are evoked antidromically, the next orthodromic impulse arises at about the time it would have if an equal number of impulses had been generated orthodromically (Murray 1965*b*). Over this time scale, the nerve is more of an integrator than the differentiator required for accommodation. For trains of brief stimuli applied to the epithelium (at frequencies greater than about 5/s) the receptor also behaves as an integrator and responds essentially to the d.c. component of the stimulus (Murray 1965*a*).

The role of the receptor cells in accommodation can be illuminated by voltage clamp experiments with repetitive pulses. The onset of the late outward current is facilitated; that is, it requires less Ca influx for some seconds after a prior stimulus that causes a Ca influx (Clusin and Bennett

1977*b*, Huse et al. 1977). The duration may be of the order of a hundred of the oscillations of the short-circuited epithelium, and comparable to the time for accommodation to weak stimuli. Careful analysis of the time course of the facilitation should illuminate the time course of removal of cytoplasmic Ca and perhaps provide insights into mechanisms that are of some wider application.

Multimodality of Responsiveness

Given the many factors involved in the electrical sensitivity of the receptors and the high degree of sensitivity, it is not surprising that the ampulla is responsive to many modes of stimulation (Murray 1974). The receptors are as sensitive to temperature as mammalian skin receptors, but no analysis is available of the site of action. The accommodation patterns indicate that this is not a simple thermoelectric effect, but temperature would be expected to act on many of the receptor mechanisms. Again, one notes that the receptors are most unfavorably situated for sensing temperature, and behavioral experiments demonstrate an electrical perception mediated by the ampullae (Kalmijn 1974, 1978). Mechanical sensitivity, although present, is unremarkable, particularly compared to the ordinary lateral-line receptors, and the evidence indicates little activation of ampullae by the usual mechanical stimuli in the animals' environment.

Sensitivity to magnetic fields is present through induced electric fields when the animal is moving, and Kalmijn gives evidence that this sensitivity is used in navigation.

Sensitivity to salinity changes is transient, if quite marked (Murray 1965*b*). Whether such responses are present as the animal swims through large gradients, or whether they require local application to the canal opening, is unknown.

Accessory Structures and Sensitivity of Electroreception

In the marine elasmobranchs the ampullary canals radiate from the several capsules in which the ampullae are located (Murray 1967, Bennett 1971*b*). The resistance of the canal walls is extremely high, so that their space constants are extremely long (Waltman 1966). The resistance of the ampullae that terminate the canals is also high compared to the axial resistance of the canals. Thus, there is little voltage drop along the canals. The resistance of the skin, in the skate at least, is quite low, and the body interior is of somewhat greater resistance than seawater. Thus, a voltage gradient in the water is not greatly distorted by the fish itself (Murray 1967).

Given these conditions, the stimulus for the ampulla is the difference between the voltage at the opening of the canal and that in the body interior just outside the basal faces of the receptors cells. The different receptors, with different lengths and orientations, would thus have different absolute sensitivities to uniform gradients of various orientations. Each receptor would be most sensitive to gradients parallel to the axis of the canal. The

dorsal canals running posteriorly are the longest, and if ampullary sensitivities are constant, these receptors would be expected to be the most sensitive. In mandibular receptors Murray (1967) observed a sensitivity of 1 to 2 μV for changes in impulse frequency detected by ear. In excised receptors from the group with long posteriorly running canals, we have observed a clear change of PSP amplitude produced by single stimuli of about 5 μV (Figure 9). The receptors of different groups therefore may be similar in voltage sensitivity. However, the posteriorly running canals are up to 20 cm long and their receptors would thereby have a tenfold greater sensitivity to voltage gradients than the mandibular receptors with canals 2 cm long. Taking the best values, we obtain a maximum sensitivity to voltage gradients of 0.1 μV/cm, which is tenfold less sensitive than the behavioral threshold of 0.01 μV/cm (Kalmijn 1974, 1978). The discrepancy could be in the experimenters' failing to find the most sensitive receptors, as well as in central processing of data from many receptors. The number of receptors with maximum canal length along a given direction is about 10 to 20, and each is innervated by about five nerve fibers. Thus, the total number of afferent inputs is quite small compared to those in visual and auditory systems.

Although a dramatic difference in threshold of receptors with different canal lengths has not been observed, the long time constants of the canal walls should produce in longer canals greater attenuation of higher frequency signals (Waltman 1966) that are in the range of responsiveness of the receptors (Murray 1967). Shorter canals could therefore not only provide more local information but also give more information about higher frequency signals.

Evolutionary Considerations

The ampullae of Lorenzini presumably function similarly throughout the elasmobranchs. Their operation must be passive in animals without electric organs. The weakly electric organs of the skates could conceivably be used in active electrolocation because their pulses are of an appropriate amplitude and duration, but because of the small size of the discharge very little is known about normal occurrence (Bennett 1971a). A communication function would also be possible and indeed seems likely, even if active electrolocation is not employed. In the torpedinids the discharge is very powerful, and the question is more one of how they prevent damage to their ampullae. One species of torpedinid, *Narcine brasiliensis*, is known to have a weakly electric organ that can generate only a low-voltage, low-frequency signal, but its normal activity is unknown (Bennett and Grundfest 1961).

The freshwater stingray *Potamotrygon* presents an interesting variant on the general elasmobranch pattern. It has long dwelled in fresh water, and as in the weakly electric teleosts, its skin is of high resistance and its ampullary canals are very short (Szabo et al. 1972, Szamier and Bennett 1971). The body interior is of low resistance compared to the surrounding medium, and the interior is essentially isopotential (Szabo et al. 1972); voltage gradients

due to external fields are developed almost entirely across the skin. Presumably this represents an adaptation to the high resistance of fresh water in that the fish is better impedance matched to its environment.

In spite of the superficial similarity to freshwater teleost receptors, *Potamotrygon* receptors should still be classed as ampullae of Lorenzini, for their receptor cells have apical cilia like those of other elasmobranch receptors and they exhibit the same polarity of sensitivity (that is, they are excited by externally negative stimuli and inhibited by externally positive stimuli (Figure 10)). The receptors have not been tested for the opposite effects of very strong stimuli that directly polarize the basal faces. Although
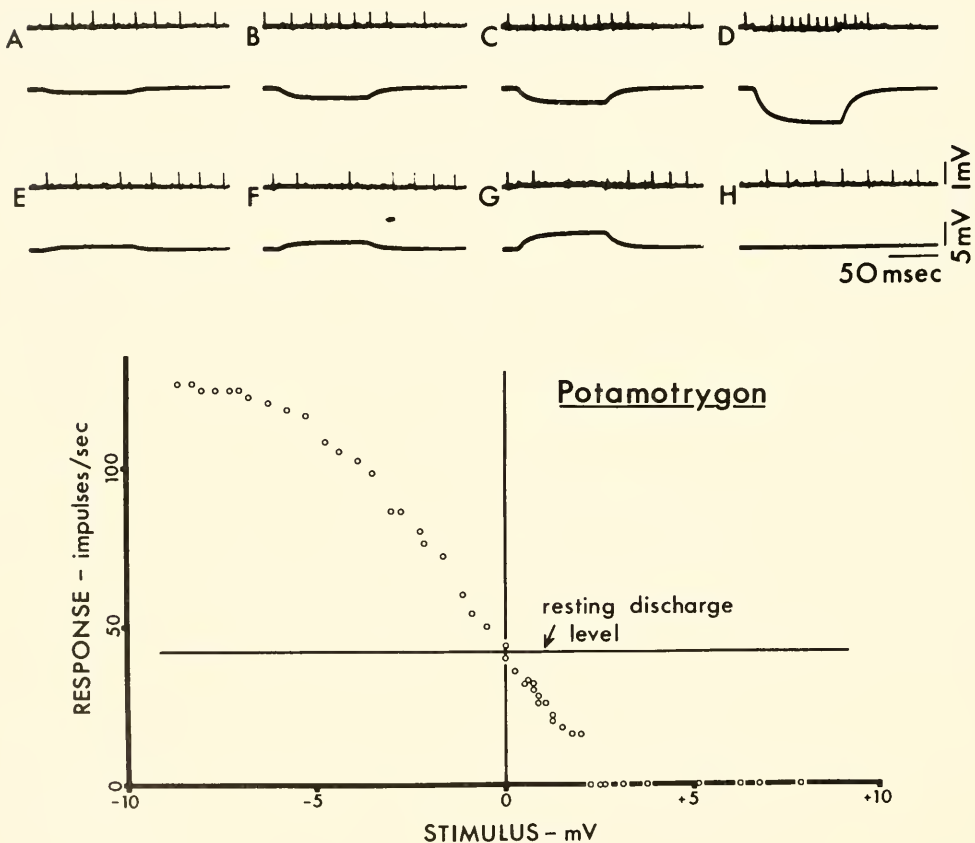
Figure 10   Responses of an afferent fiber from an ampulla of Lorenzini of the freshwater stingray *Potamotrygon*. Upper trace was recorded from a single afferent fiber, lower trace represents potential produced on the skin (in air) outside the receptor by a square pulse of current. High resistivity is indicated by the gradual rise and fall of potential. Outside negative stimuli excite (A-D) and increase the impulse frequency over that of the tonic resting discharge (H). Outside positive stimuli inhibit (E-G). The graph shows the mean discharge frequency during 100-ms pulses of varying amplitudes. A sigmoidal relation, linear for small stimuli of either sign, is evident. Compared to that of marine (skate) ampullae, the sensitivity is very low.

the canals are quite short, no response has been recorded on the skin surface. Innervation of each ampulla is by several fibers.

A further similarity between *Potamotrygon* and freshwater teleosts is in sensitivity to stimuli in the millivolt range. This may represent an adaptation to a greater ambient electrical noise in the freshwater than in the marine environment (Hopkins 1973). The influence of marine and freshwater environments is further attested to by the marine catfish *Plotosus* (Obara 1974, 1976). This fish has developed long canals like those of the marine elasmobranchs. Also, its receptors are very sensitive and generate action potentials as skate receptors do. However, the receptor cells lack a cilium, and the polarity of sensitivity is the same as that in other teleosts and opposite to that in elasmobranchs. Innervation is multiple, as in elasmobranchs. The comparative data indicate that the marine environment selects for long canals and high sensitivity while the freshwater environment does the opposite. The association of high sensitivity with action potential generation in separately evolved marine fishes supports the hypothesis that greater sensitivity is achieved by an active response mechanism.

The sturgeon is a primitive, bony fish that also has electroreceptors (Teeter and Bennett 1976). These receptors in the freshwater shovel-nose sturgeon have almost no canals, but they show both apical cilium on their receptor cells (R. B. Szamier, unpublished observations) and the polarity of sensitivity of the elasmobranchs. It seems, therefore, that these receptors are homologous to those of the elasmobranchs and should be classified as ampullae of Lorenzini. The teleost receptors may well have arisen independently in the weakly electric mormyrids, in the gymnotids, and in catfish, but some change in the teleost line appears to have led to their similarity to one another and difference from the elasmobranchs. Investigation of other primitive fishes may further support this hypothesis.

ACKNOWLEDGMENTS

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ELECTRIC AND MAGNETIC SENSORY WORLD OF
SHARKS, SKATES, AND RAYS

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As sharks, skates, and rays base their actions on the physical stimuli they receive from the oceanic environment, a thorough knowledge of their sensory abilities is necessary to a full appreciation of the behavior of these ancient fishes. Certainly, underwater light, sound, and odor fields are quite different from those on land. Moreover, elasmobranchs respond also to weak electrical voltage gradients, which they detect with the ampullae of Lorenzini. Thus, electroreception adds another, unique dimension to the sensory world of the elasmobranch fishes.

The biological significance of the animals' electrical sensitivity first became evident in behavioral studies in which sharks, skates, and rays were observed to cue in on bioelectric fields emanating from prey. Elasmobranchs may also sense the electric fields of ocean currents flowing through the earth's magnetic field, and use this faculty to follow the currents during migration, as a daily means of orientation, and to compensate for passive drift (passive electro-orientation). Eventually, by swimming through the earth's magnetic field, the animals induce electric fields that may enable them to establish their compass headings (active electro-orientation). Marine stingrays have shown their ability to orient with respect to the earth's magnetic field in recent training experiments.

After a short review of the studies that led to the discovery of the elasmobranchs' electric sense, this article describes new field observations on the electrical aspects of predation and experiments on captive sharks and rays involving geomagnetic orientation. For the morphology, physiology, and physics of the receptor system, the reader is referred to Bennett and Clusin (1978), Murray (1974), and Kalmijn (1974).

THE DISCOVERY OF AN UNCONVENTIONAL SENSORY MODALITY

Studying the sensory behavior of the North American catfish *Ictalurus nebulosus*, Parker and Van Heusen (1917) observed blindfolded specimens react to the approach of metallic rods at distances of several centimeters, whereas a glass rod did not elicit a noticeable response until it actually touched the animals' skin. Through successive elimination of the physical stimuli emanating from the metallic rods, the investigators convincingly demonstrated that the distant responses were due to galvanic currents generated at the interface between metal and aquarium water. On the verge of revealing the electric sense of freshwater catfish, they nevertheless failed to realize the biological implications of their novel finding.

In 1934, Dijkgraaf also noticed a great sensitivity to metallic objects in the dogfish *Scyliorhinus canicula*, a small, bottom-dwelling shark of the Mediterranean and coastal Eurpoean waters. A quarter of a century later, Kalmijn confirmed the electrical nature of the shark's response and undertook to investigate its biological significance. (The results of both studies were reported in Dijkgraaf and Kalmijn's joint paper of 1962.) Previously, Lissmann had discovered that the African and South American

mormyrid and gymnotid fishes produce weak electrical discharges to actively probe their environments (Lissmann 1951, 1958). However, which fields the nonelectric sharks and catfish might detect in their surroundings remained an open question at the time.

Subsequent research at the University of Utrecht, the Netherlands, soon disclosed that marine elasmobranchs are indeed remarkably sensitive to weak electric fields (Kalmijn 1966). The author established transient cardiac decelerations upon application of uniform electric fields by recording the heartbeat of free-swimming skates (*Raja clavata*) with permanently implanted electrodes. The skates showed their cardiac reflex down to voltage gradients as low as 0.01 μV/cm, thus exhibiting the highest electrical sensitivity known in the animal kingdom. In later behavioral tests, sharks and skates appeared most responsive to frequencies in the range from 0 (direct current) to about 8 Hz (Kalmijn 1971, 1974). The receptors detecting these weak, low-frequency electric fields are the ampullae of Lorenzini, delicate sensory structures in the elasmobranchs' protruding snouts (Murray 1962, Dijkgraaf and Kalmijn 1963) (Figure 1).

Measuring the electric fields in the laboratory habitat of the sharks and skates, I found that aquatic animals produce direct-current (d.c.) and low-frequency voltage gradients in the water, which mainly stem from potential differences at their skin–water interfaces (Kalmijn 1966, 1969,
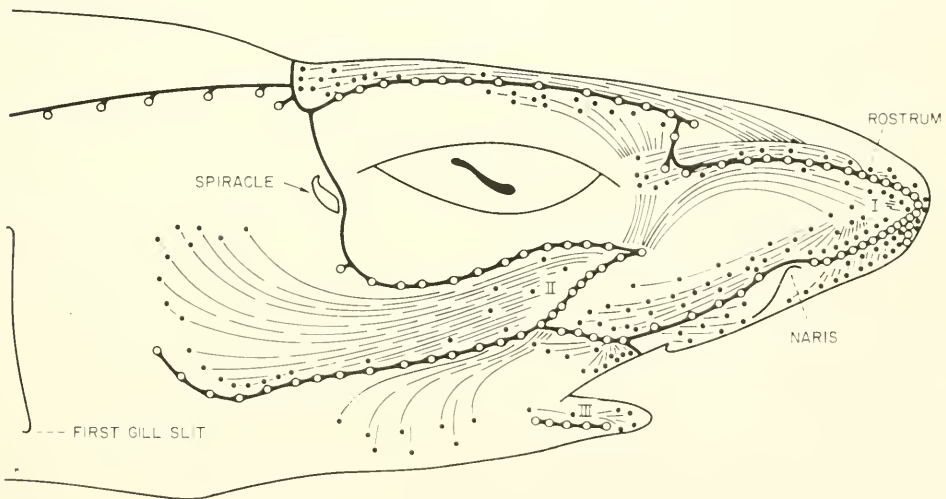
Figure 1 Lateral-line canals and ampullae of Lorenzini in the head of the shark *Scyliorhinus canicula*. The lateral-line canals (in heavy black) contain the mechanoreceptive neuromasts. They connect to the outside through linearly arranged skin pores (open circles). The openings of the Lorenzinian ampullae (solid dots) form a more dispersed pore pattern. Each gives access to an often long, jelly-filled canal (broken lines) ending in a blind sensory swelling. The ampullae proper are clustered together in a rostral (I), an infraorbital (II), and a small mandibular (III) capsule. By the technique of selective denervation, the ampullae of Lorenzini were shown to be the receptors responsible for the shark's behavioral reactions to weak electric fields (Dijkgraaf and Kalmijn 1963).

1974). In fish, for example, the mucous membranes lining the mouth and the gill epithelia in the pharynx give rise to steady d.c. fields, usually modulated by ventilatory movements. Externally, the bioelectric fields are of a distributed-dipole configuration and accordingly fall off steeply with increasing distance. Yet, the voltage gradients in the vicinity of small fish and wounded crabs often measured over 0.01 μV/cm at distances up to 25 cm. As these fields exceeded the elasmobranch threshold sensitivity, they strongly suggested the possibility of electrical prey detection.

To substantiate the electrical aspects of predation, I decided to analyze the feeding responses of the shark *Scyliorhinus canicula* and the skate *Raja clavata* to small specimens of the flounder *Pleuronectes platessa* (Kalmijn 1971). The prey fish was carefully introduced into the seawater habitat, and after it had hidden itself in the sand a few drops of whiting extract were spread diffusely throughout the water. Aroused by the odor, the sharks and skates began within seconds to haphazardly search the bottom of the pool. When they came within 10 to 15 cm of the flounder, they made well-aimed dives at the prey, uncovered it from under the sand, and devoured it voraciously (Figure 2A). Next, I enclosed the flounder in a flat agar chamber to conceal the prey visually, chemically, and mechanically without impeding its bioelectric field. (Dissolved in seawater, 3- to 4-percent agar makes a rigid structure that is virtually transparent to electrical current.) The agar chamber was placed on the bottom of the pool, just under the surface of the sand. To keep the flounder alive, the chamber was ventilated with a steady flow of seawater. Despite these changes, the sharks and skates again made well-aimed attacks from the same distance and in the same frenzied manner as when no agar was screening the prey (Figure 2B). These results were in full accord with the assumption that sharks and skates can locate prey bioelectrically.

To prove that the 1-cm-thick roof of the agar chamber presented an adequate barrier to odor stimuli, I substituted cut pieces of whiting for the live flounder. The whiting formed the regular diet of the sharks and skates, and was kept frozen for several days before use. This time, though strongly motivated by the odor of the seawater flow ventilating the chamber, the predators did not respond at all to the food when swimming over the agar structure (Figure 2C). Then, I put the flounder back in the chamber and covered the whole structure with a very thin, electrically insulating film of polyethylene. Under these circumstances, the sharks and skates did not respond to the live prey either, though they eagerly searched and often passed right over it (Figure 2D). This dramatic effect could not conceivably

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Figure 2 In feeding, the shark *Scyliorhinus canicula* cues in on the bioelectric field of its prey. (A) Motivated by the diffuse odor of fish extract, *Scyliorhinus* shows well-aimed attacks at a small flounder (*Pleuronectes platessa*) hiding in the sand (solid arrow). (B) With the flounder enclosed in an agar chamber to conceal the fish visually, chemically, and mechanically without impeding its bioelectic field, *Scyliorhinus* continues to dive at its prey (which is kept alive by ventilating the chamber with a flow of seawater: dashed arrows). (C) However, with cut pieces of fish instead of the live

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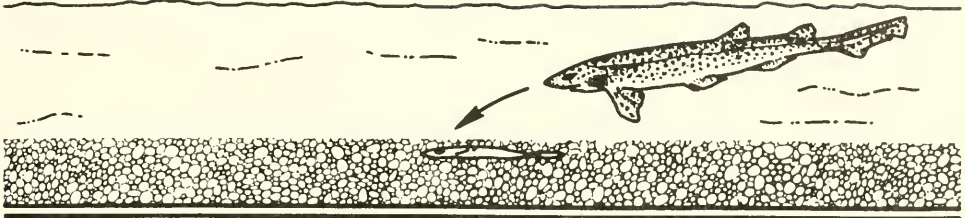
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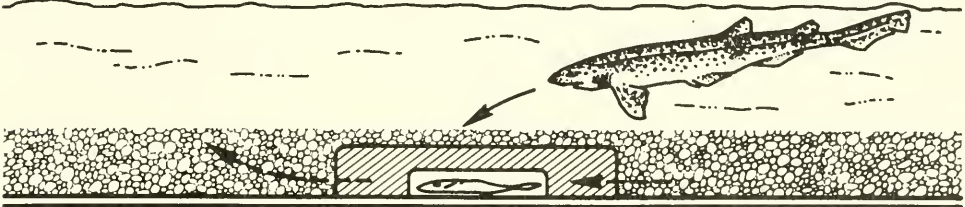
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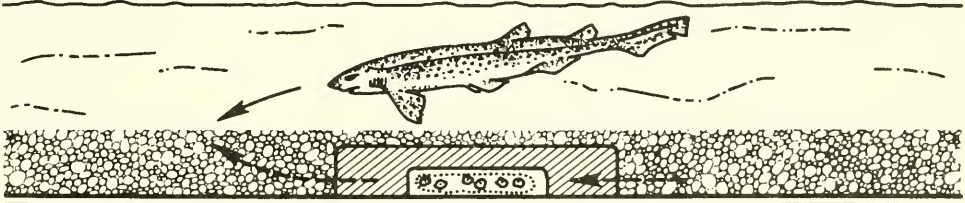
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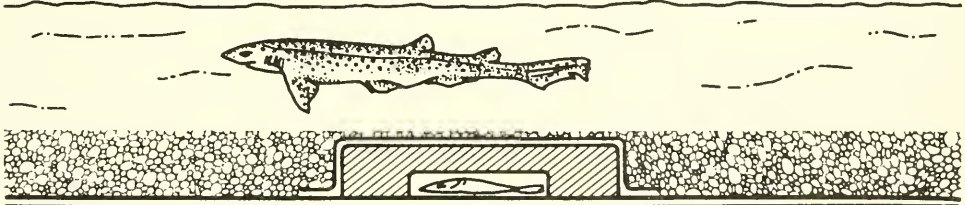
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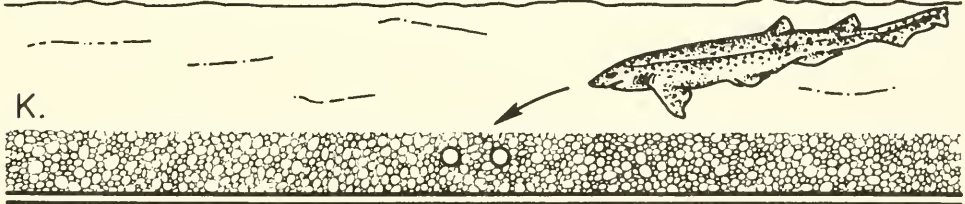
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flounder, *Scyliorhinus* no longer aims directly at its food in the agar chamber, but searches the area where the seawater flow comes up through the sand. (D) When the chamber is covered with a thin polyethylene film, which is mechanically negligible compared to the stiff agar roof, but offers an extremely high electrical resistance, *Scyliorhinus* does not detect the live flounder any longer either. (E) Eventually, when the flounder's bioelectric field is simulated by passing a weak electrical current between two electrodes buried in the sand, *Scyliorhinus* responds to the source of the field from the same distance and with the same vigor as if it were the actual prey (Kalmijn 1971).

be due to the mechanical properties of the polyethylene film, as even the stiff agar roof to which the film was added failed to appreciably weaken the feeding response. Hence, the sharks and skates had not detected the agar-screened prey merely by visual, chemical, or mechanical cues.

By the same token, the all-or-none effect of the polyethylene film was fully in line with the hypothesis of electrical prey detection. The film offered an extremely high electrical resistance, whereas the agar layer did not distort the flounder's bioelectric field to any extent. To provide direct evidence, however, I simulated the presence of the flounder by passing an electrical current of biological strength between two salt-bridge electrodes hidden in the sand. After odor motivation, the sharks and skates, as expected, displayed the same characteristic feeding behavior with respect to the electrodes (whether or not covered with agar) as they did to the actual prey (Figure 2E). They dug tenaciously at the source of the field, responding again and again when coming across the electrodes. Similar results were obtained in the leopard shark (*Triakis semifasciata*), the lemon shark (*Negaprion brevirostris*), the smooth dogfish (*Mustelus canis*), the thornback (*Platyrhinoidis triseriata*), the small skate (*Raja erinacea*), the round stingray (*Urolophus halleri*), and the freshwater stingray (*Potamotrygon circularis*) (Kalmijn 1974, in Szabo et al. 1972, and unpublished). Thus, these experiments not only established the elasmobranch's electric sense, but also signified its great biological importance in the animals' daily life.

FIELD OBSERVATIONS ON CAPE COD SHARKS

The earlier experiments were all performed on captive sharks, skates, and rays in polyvinyl and fiberglass pools under well-controlled laboratory conditions. In the ocean, the situation is more complex, as the electric fields of prey mingle with those of physical and chemical origin (Kalmijn 1974). Therefore, the author (1977a,b) has sought to verify the laboratory results in tests on wild specimens roaming freely in their natural habitat. It appeared very difficult, however, to approach the animals without introducing into the environment galvanic fields or other perturbations likely to interfere with the fish's normal behavior. After all, the electrical sensitivity of sharks and catfish had first manifested itself in the animals' unusual responses to metallic objects in contact with the water.

During the summer of 1976, I learned from longline fishing off Cape Cod, Massachusetts, that the smooth dogfish (*Mustelus canis*) regularly frequents the shallow, inshore waters of Vineyard Sound on its nightly feeding excursions. This predatory shark is a warm-season visitor arriving at Woods Hole in May and leaving for the South again in late October or shortly thereafter. It is an active bottom hunter, preying on small fish as well as crustaceans and other invertebrate animals. The females reach an average length of 1.15 m; the males are somewhat smaller. The smooth dogfish is truly viviparous; the newborn measure 29 to 37 cm (Bigelow and Schroeder 1953).

To observe the sharks' feeding behavior, I went to sea with my student collaborators in an inflatable rubber raft (Zodiac Mark II) free of any metal under the waterline. On station in 2.5- to 3.0-m-deep water over a sand patch devoid of weeds, we attracted the sharks by pumping small amounts of liquefied herring through a long Tygon tube (7.9 mm I.D.) that ran from the raft to the bottom of the sound (Figure 3). The chumming tube was attached to a polypropylene line 7.9 mm in diameter, suspended from a Styrofoam float and stretched over the ocean floor between two polyvinyl pipes anchored in low-profile cinder blocks. Starting after dark, we illuminated the area with a 100-W, battery-operated, underwater light. To look from the surface down into the water, we used a glass-bottomed viewing box secured behind the stern of the raft.
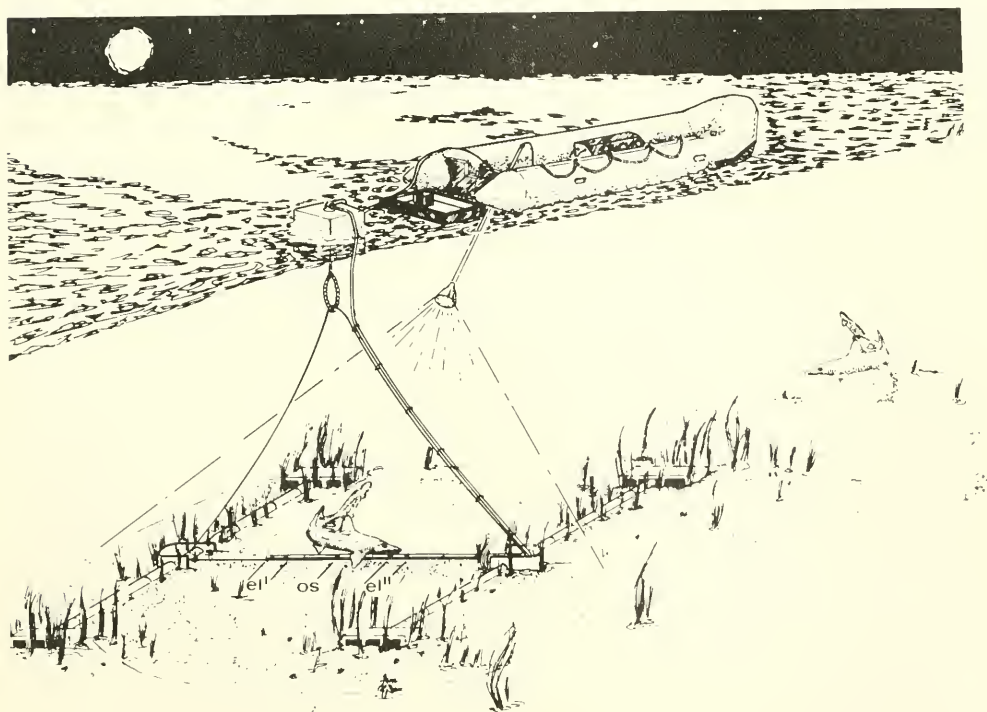
Figure 3 Electrical prey detection in the shark *Mustelus canis:* field setup in Vineyard Sound, Massachusetts. After dark, the sharks' feeding behavior is observed from an inflatable rubber raft with the aid of a glass-bottomed viewing box and a sealed-beam underwater light. The sharks are attracted and motivated to search for food by the odor of fish extract that exudes from a chumming tube running from the raft to the bottom of the sea. A weak electrical current is passed between two electrodes—either the ones to the left (el′) or the ones to the right (el″) of the odor source (os)—to mimic the bioelectric field of small prey, the other pair functioning as the control. Instead of biting at the opening of the chumming tube, the sharks turn sharply toward the current-carrying electrodes (el″) to attack the electrically simulated prey. (From Kalmijn 1977b.)

To present the sharks with electrically simulated prey, two pairs of seawater salt-bridge electrodes were tied to the polypropylene line and positioned on the sand, one on either side of the odor source and 25 cm from it (Figure 4). Mecca underwater plugs with stainless steel pins and integral cables connected the seawater-agar content of the 30- to 90-cm-long Silastic salt-bridge tubes (3.2 mm I.D.) to the electrical stimulator set up in the rubber raft. The use of a constant-current source and salt-bridge electrodes practically eliminated the adverse effects of polarization at the stainless-steel/seawater-agar interfaces. From the raft, we could conveniently vary the strength of the field and select the pair of electrodes to be energized while the other pair functioned as the control. The applied d.c. dipole moments ranged from 1 to 8 μA $\times$ 5 cm (dipole current $\times$ distance between electrodes), approximately corresponding to the bioelectric fields of small prey at a seawater resistivity of 20.0–21.5 $\Omega \cdot$ cm and a temperature of 20–22°C (Kalmijn 1971).

After entering the test area, the dogfish began randomly searching the sand, evidently trying to locate the odor source. Both young and mature sharks were observed, sometimes alone, sometimes in groups of two to five. Neither the raft nor the underwater light appeared to disturb them. Most interestingly, when nearing the underwater setup, the animals did not bite at the opening of the chumming tube, but turned sharply toward the current
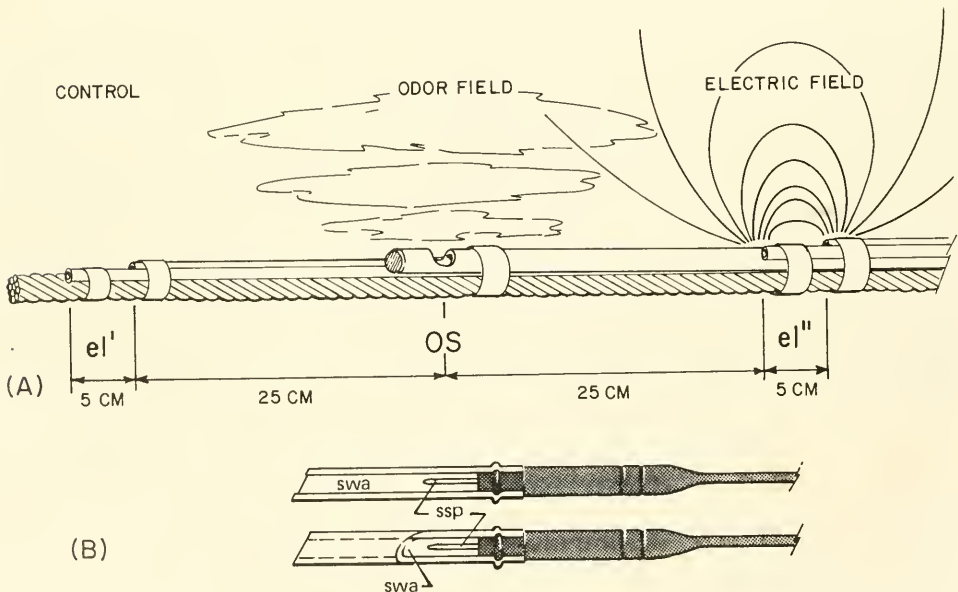
Figure 4   (A) Polypropylene line with linear array of chumming the tube and salt-bridge electrodes. Plastic markers indicate the positions of the odor source (os) and electrode openings (el' and ell''). (B) Two of the underwater plugs connecting the salt-bridge tubes to the electrical cables. The stainless-steel pins (ssp) are in direct contact with the seawater-agar content (swa) of the salt-bridge tubes.

electrodes from distances up to 25 cm, viciously attacking the electrically simulated prey (Figure 5). After snapping the line with their teeth at the position of the electrodes, the sharks usually attempted to rip them apart;
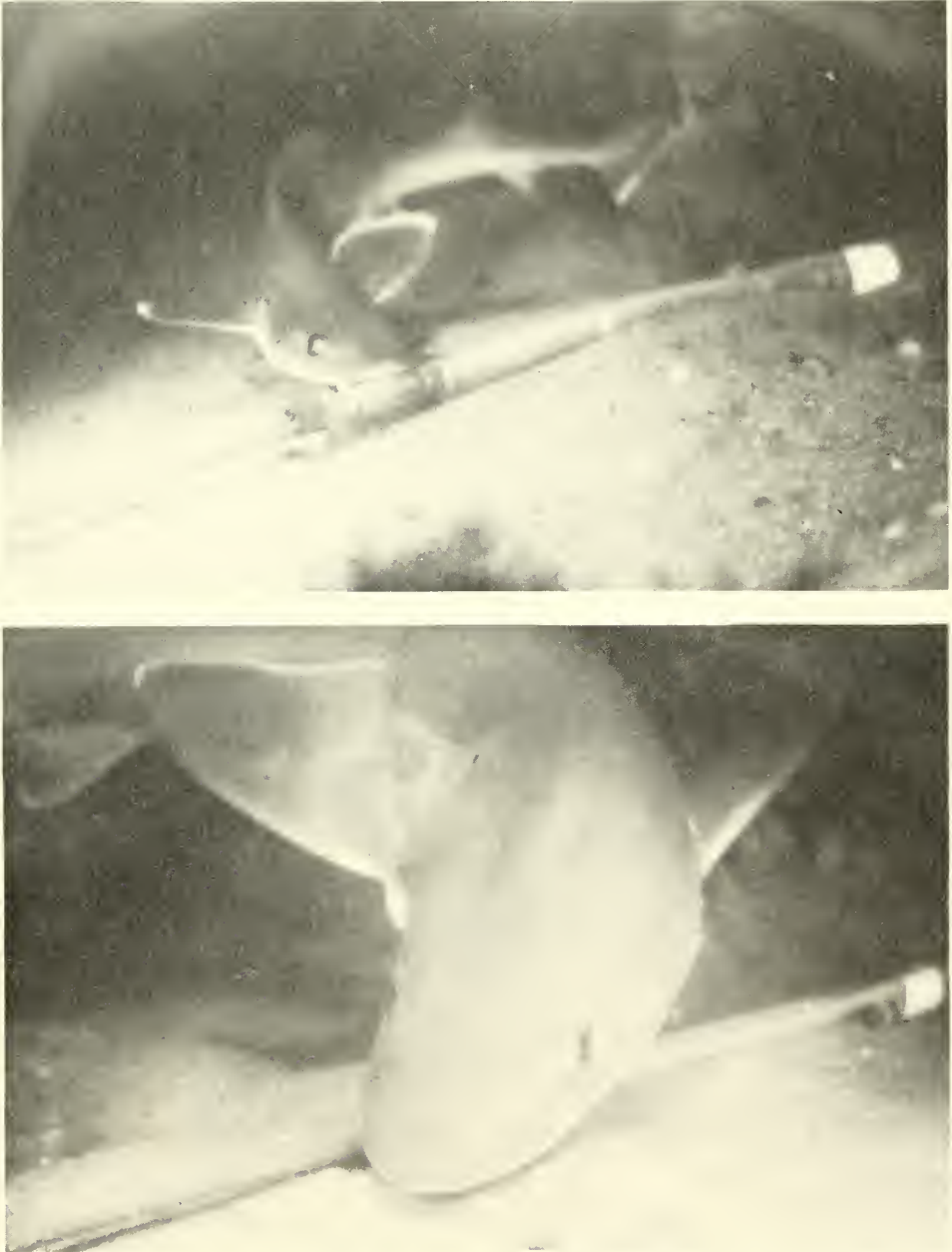
Figure 5 (A) Young and (B) adult specimens of the shark *Mustelus canis* biting at electrically simulated prey. Light band, odor source; dark bands, electrodes. Photographs taken in a large indoor aquarium by Seth W. Rose.

one night they succeeded. When the current was switched to the other pair of electrodes, the animals would let go, circle about for a while, and strike again—this time at the electrodes on the other side of the odor source. Over the summer, we recorded several hundred attacks, many of them in double-blind fashion, leaving no doubt as to the validity of our observations. At lower current levels the sharks kept responding, though from increasingly shorter distances.

These new field data clearly demonstrate that odor-motivated sharks can detect and take prey by the exclusive use of their keen electric sense, not only under favorable laboratory conditions, but also in their electrically more intricate oceanic milieu. This, of course, does not imply that other sense organs, such as the acustico-lateralis system, may not also play an important part in nocturnal predation (cf. Myrberg 1978). Although in our studies odor cues attracted the sharks from a distance, at short range the bioelectric fields proved much more compelling to the animals. When following an odor trail, the sharks obviously search for more precise information to accurately locate their prey and, in the eventual attack, home in on their victim's bioelectric field to seize it unerringly with one quick move.

Behaviorally, elasmobranchs respond most readily to d.c. fields. Electrophysiologically, however, the ampullae of Lorenzini are not true d.c. receptors, though they do detect frequencies as low as 0.1 Hz (Murray 1962). That is, to sense the prey's d.c. field, elasmobranchs must move with respect to their prey. Alternatively, they may detect the low-frequency components that accompany the prey's ventilatory, fin, and body movements. In fact, aroused by odor, sharks and skates zero in on d.c. as well as low-frequency dipole fields of biological strength (Kalmijn 1971). The behavioral frequency range corresponds well with the array of biological stimuli, the physiological properties of the sense organs, and the physical characteristics of the accessory structures (Murray 1962, Waltman 1966, Kalmijn 1974). Furthermore, independent of the angle of approach, the elasmobranchs aim directly at their prey, evidently deriving its location from the spatial configuration of the animal's bioelectric field.

Besides sharks, other fish, among them the American eel *Anguilla rostrata*, visited the test site. The eels often nibbled at the opening of the chumming tube, but they paid no attention to the current-passing electrodes. Their behavior was particularly noteworthy, since the American eel had been reported to exhibit transient cardiac decelerations when subjected to weak electric fields (Rommel and McCleave 1972, 1973) similar to those observed in elasmobranch fishes (Kalmijn 1966). The eels' responses, however, have not been independently confirmed (Enger et al. 1976), nor are these fish known to have specific electroreceptors (Leonard and Summers 1976). In predation, they evidently rely more on chemical cues. On the other hand, the catfish *Ictalurus nebulosus* again attacked the prey-simulating electrodes when tested in a local freshwater pond (at dipole moments of 0.5 to 4 μA $\times$ 1 cm at 19 kΩ · cm).

For the 1977 season, we have outfitted a modified fiberglass 21-ft Boston Whaler for behavioral studies on the electrical sensory performances of not

only the shallow-water, bottom-dwelling sharks, but also the more open-ocean forms off Cap Cod, in particular the blue sharks, *Prionace glauca*. The Whaler will in addition serve as a nongalvanic working platform for exploring the d.c. and low-frequency electric fields in the animals' natural habitat. Thus, our oceanic endeavors have opened up new avenues expected to lead to a better understanding of the electrical sensory world of the marine elasmobranch fishes.

Although we conceived our field work mainly with the undisturbed oceanic environment of the sharks in mind, our test results also indicate that attacks on humans and underwater gear may be elicited and guided by electric fields resembling those of regular prey. The human body, especially when the skin is damaged, creates in the water d.c. bioelectric fields that sharks may detect from distances of up to 1 or 2 m (Kalmijn 1971). The galvanic fields of metallic objects on the body are often even stronger. In this connection, the U.S. Navy's antishark screen, which consists of a large polyvinyl bag suspended from an inflatable flotation collar and was designed (by Dr. C. Scott Johnson at the Naval Undersea Research and Development Center, San Diego, California) to visually and olfactorily conceal a mariner in distress, looks promising from an electrical point of view as well. Sidetracking the sharks to alternative sources of electricity that secondarily release a discouraging agent might, under certain circumstances, be another means of warding off electrically evoked shark bites.

It should be emphasized—whether our interest in the animals is purely academic or more practical—that in behavioral studies of elasmobranch and other electrosensitive fishes the electrical aspect of the environment must be taken into account. In land facilities, the background fields should be well controlled; in the ocean, they should preferably be left undisturbed. Any metallic device lowered into the water is likely to produce fields strong enough to disturb the animals' electromagnetic environment. Fields of biological strength may trigger false responses; stronger fields may first scare the animals, but will soon cause them to ignore electric fields altogether and for the moment deprive them of their electrical sensory abilities (Kalmijn, unpublished). If recognized, these problems can, with proper care, be overcome. Thus, the author's technical efforts have yielded a first insight into the electrical sensory world of the elasmobranch fishes.

EXPERIMENTAL EVIDENCE OF GEOMAGNETIC ORIENTATION

With such a marvelous sensory system enabling sharks, skates, and rays to cue in on the bioelectric fields of their prey, one wonders what other underwater voltage gradients elasmobranch fishes might detect and use. Of the various fields in the marine environment, I have more recently been concentrating on those predicted by Faraday (1832) in his historic lecture on electromagnetic induction for they show great potential as a means of open-ocean orientation and navigation. In biological terms, when cruising

through the earth's magnetic field, sharks, skates, and rays induce electric fields that depend on the directions in which they are heading. Because the induced voltage gradients are well within the dynamic range of the elasmobranchs' highly sensitive electroreceptor system, they may constitute the physical basis of an electromagnetic compass sense (Figure 6). Since in this instance the fish themselves generate the fields they detect, this is a form of active electro-orientation.

I have elaborated on the physical principles of electromagnetic orientation in previous papers (Kalmijn 1973, 1974). Here, I will only briefly review the special case of active electro-orientation (Figure 7A). When the fish swims to the east and thus crosses the horizontal component of the earth's magnetic field, it generates—according to Faraday's law—an internal, dorsoventral emf of induction

$$\int_{V}^{D} (\mathbf{v} \times \mathbf{B}_h) \cdot d\mathbf{s},$$

with v the swimming velocity, $\mathbf{B}_h$ the horizontal component of the earth's magnetic induction, and s any internal path from the ventral (V) to the dorsal (D) surface of the animal. The induced emf gives rise to electrical currents that flow ventrodorsally through the moving fish and loop back through the stationary environment (with respect to which the motion takes place).
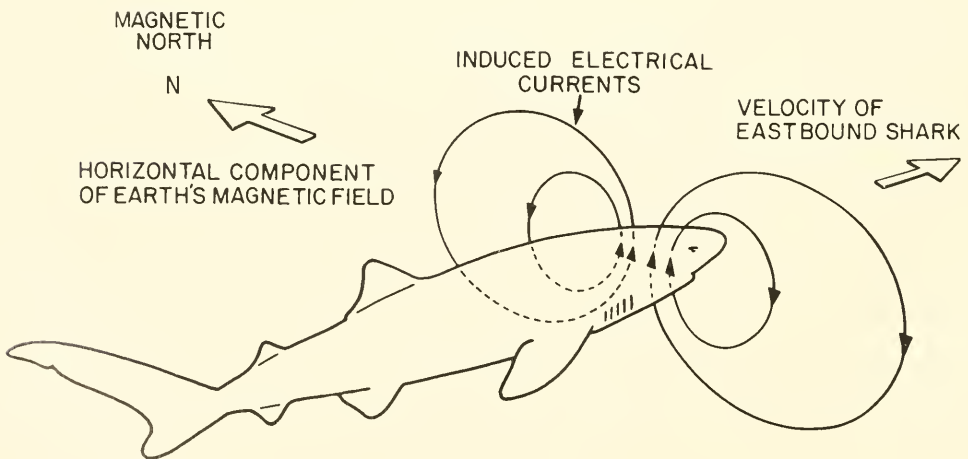
Figure 6 A shark swimming through the earth's magnetic field induces electric fields that provide the animal with the physical basis of an electromagnetic compass sense. (From Kalmijn 1974.)

Within the fish, these currents lead—according to Ohm's law—to a dorsoventral ohmic potential difference

$$\int_V^D -\rho \mathbf{J} \cdot ds,$$

with ρ the resistivity of the tissues, $\mathbf{J}$ the current density, and the minus sign indicating that it opposes the induced emf. Thus, the total dorsoventral potential difference (the induced emf plus the ohmic voltage drop) equals

$$\int_V^D (\mathbf{v} \times \mathbf{B}_h - \rho \mathbf{J}) \cdot ds.$$

However, because of the relatively large volume and low resistivity of the seawater, the fish is electrically almost completely short-circuited. That is, the ohmic potential difference effectively counteracts the induced emf, or

$$\int_V^D -\rho \mathbf{J} \cdot ds$$

approaches

$$\int_V^D (\mathbf{v} \times \mathbf{B}_h) \cdot ds,$$

and the total potential difference between the dorsal and ventral receptor pores

$$\int_V^D (\mathbf{v} \times \mathbf{B}_h - \rho \mathbf{J}) \cdot ds$$

becomes negligibly small. On the other hand, the ampullae of Lorenzini move through the earth's magnetic field with the same velocity as the fish, inducing along their jelly-filled canals internal emfs of

$$\int_{pore\ 1}^{amp\ 1} (\mathbf{v} \times \mathbf{B}_h) \cdot ds_1$$

and

$$\int_{amp\ 2}^{pore\ 2} (\mathbf{v} \times \mathbf{B}_h) \cdot ds_2$$

for the ventral and dorsal sense organs, respectively. Since the ampullae of
Lorenzini act as high-ohmic voltmeters (Waltman 1966; Kalmijn 1974),
there is practically no flow of current through their highly conductive
canals, and the emfs induced between the skin pores and the blind ampullary
endings develop without appreciable ohmic loss. Hence, across the sensory
epithelia forming the bases of the ampullae proper, potential differences close
to

$$- \int_{pore\ 1}^{amp\ 1} (v \times B_h) \cdot ds_1$$

and

$$- \int_{amp\ 2}^{pore\ 2} (v \times B_h) \cdot ds_2$$

appear. When the fish turns north or south, the potentials vanish; when the
fish turns west (instead of east), potentials of opposite polarity are induced
(Figure 7B). As these potentials are detectable to the elasmobranchs at
calculated swimming speeds as low as 2 cm/s, they prove the feasibility of
the proposed electromagnetic compass sense. Note that through interaction
with the vertical component of the earth's magnetic field the fish also induce
motional electric fields parallel to their transverse body axes. By additionally
sensing these fields with the laterally oriented ampullae of Lorenzini, the
elasmobranchs may determine the magnetic latitude of their position on the
globe as well.

     The first and simplest magnetic tests were performed on the leopard shark
(*Triakis semifasciata*) in outdoor fiberglass pools at the Scripps Institution of
Oceanography, La Jolla, California, in collaboration with Dr. Theodore H.
Bullock. With the fish steadily swimming along the circumference of their
circular habitat, we introduced a local magnetic field into the water by
passing an electrical current through a small induction coil (20 cm in
diameter) held outside the tank. The field was turned on when the animals
were at the far side of the pool. At that distance, the magnetic strength was
too low for the sharks to respond, and they quietly continued their lap.
Seconds later, however, on swimming into the region of the coil, the sharks
suddenly turned away from the imposed field and veered off to the center of
the tank, even though the coil did not distort the earth's ambient magnetic
field by more than 25 percent (Kalmijn 1973).

     Next, I noticed each morning the leopard sharks rest at a particular
location along the circumference of the tank in a sector a little off magnetic
north. To eliminate the possibility of visual orientation, we covered their
7-m pool with a large sheet of black plastic. We also took all experimental
structures out of the water, rotated the whole setup, and even moved it (for

other reasons) to another site. Surprisingly, this did not change the sharks'
early-morning preference. However, when we roughly neutralized the earth's
ambient magnetic field with two large coils mounted to the outside of the
tank, the animals apparently lost their sense of position and distributed
themselves randomly.
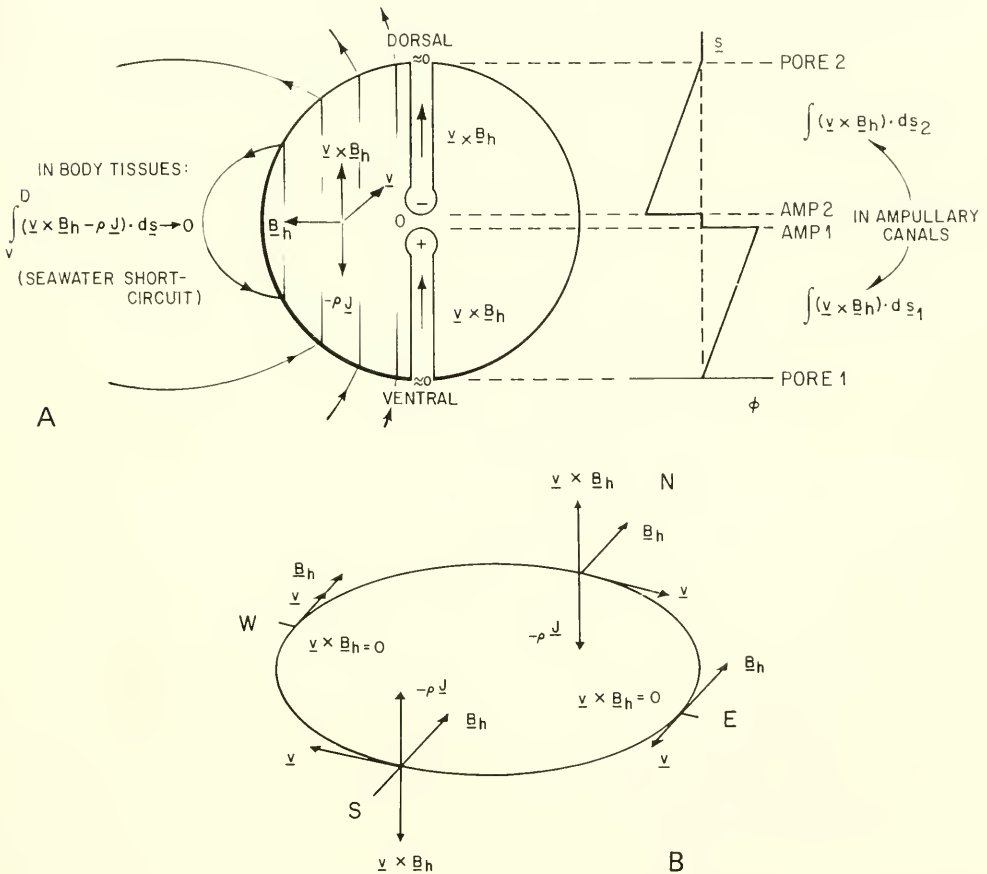
Figure 7 (A) Cross section of a shark heading east with a velocity v in a magnetic field
of horizontal induction B_h. In the shark, a ventro dorsal voltage gradient v $\times$ B_h is
induced. This in turn causes an electrical current of density J to flow: ventro-dorsally
within the *moving* fish and back through the *stationary* environment. With ρ the
resistivity along its path, the current develops an ohmic voltage gradient $-\rho J$ opposing the
induced voltage gradient v $\times$ B_h. Since the fish is virtually short-circuited by the highly
conductive seawater environment, the average $-\rho J$ of the body tissues effectively
counteracts the v $\times$ B_h, and the dorso-ventral potential difference tends to zero. In the
blind ampullae of Lorenzini, however, $-\rho J$ is negligibly weak. That is, v $\times$ B_h remains
uncompensated and, integrated along the ampullary canals, gives rise to the pertinent
electrical stimuli across the sensory epithelia that form the bases of the ampullae proper.
(B) Successive stages of a shark circling through the compass directions. (After Kalmijn
1974.)

Although their outcome was consistent with the proposed geomagnetic orientation, neither of these tests was fully conclusive. The avoidance reactions proved the sharks' sensitivity to fields of geomagnetic strengths, but were not of obvious biological significance. The animals' homing tendency was biologically more interesting, but at the time we were not technically prepared to reverse the field to randomize any remaining alternative cues, and thus to verify the magnetic nature of the response. Though preliminary, these early observations did encourage me to pursue this new line of research.

After moving to Massachusetts, I constructed new, specifically designed magnetic facilities on the Quissett campus of the Woods Hole Oceanographic Institution. To scale down the technical problems of controlling the ambient magnetic field, I looked for a good experimental animal of smaller size, which one of my students found in the round stingray, *Urolophus halleri* (Figure 8). The round stingray is a hardy, alert, and very lively elasmobranch of subtropical and tropical seas, reaching an average fin span of 25 cm. We selected specimens of only 15 to 20 cm to fit our tanks. To establish their magnetic abilities, we trained them to seek reward and avoid punishment at positions predetermined by the direction of the earth's magnetic field (Kalmijn 1977a,b).
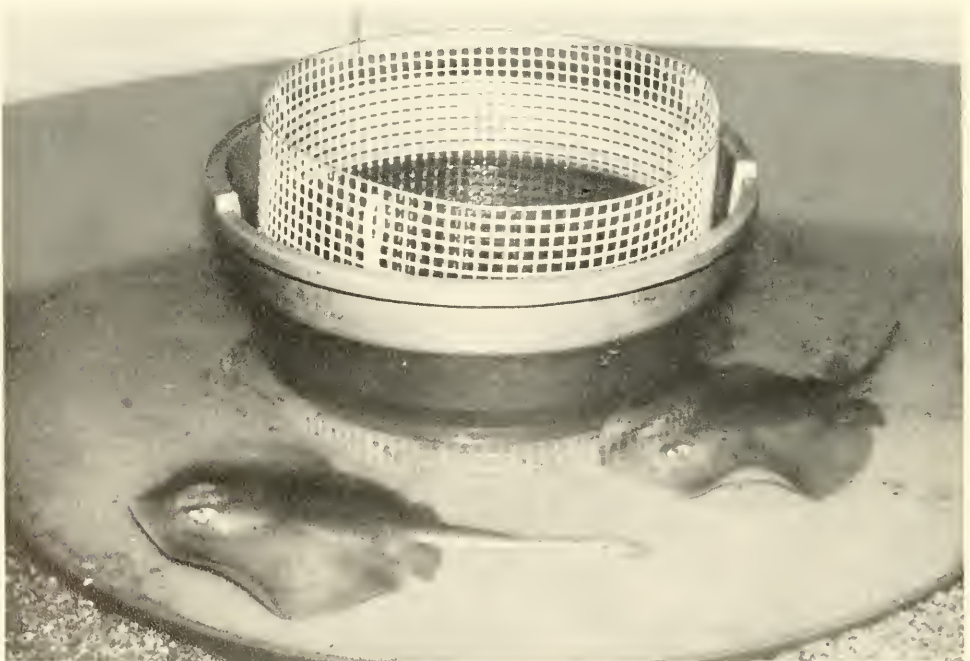
Figure 8   The two stingrays *Urolophus halleri* that learned to secure food from a plastic tub in the magnetic east and to avoid a similar enclosure in the magnetic west of their tank. The enclosures have their gates facing the wall (cf. the diagram of Figure 10). Inside screens with openings of their own can be rotated to open or close the gates.

The stingrays were tested in a circular fiberglass pool, surrounded by a light-tight, 12-sided hut devoid of ferromagnetic materials (Figure 9). The pool measured 1.8 m in diameter and rested on Teflon blocks to insulate it from ground. It was filled with natural seawater to a depth of 15 cm. Coarse sand covered the bottom, and two air-stones maintained a slow internal circulation. The seawater was kept at 20°C by regulating the air temperature in the hut. To control the horizontal component of the earth's magnetic field in the tank, two north-south oriented Helmholtz coils, each 5 m in diameter, were erected outside the hut. We chose for our experiments the horizontal induction of the Southern California region from which the animals were taken (0.26 G).

During the 1- to 2-h training sessions, twelve concealed incandescent lights illuminated the ceiling over the experimental tank to produce an even, low-level light distribution. For the rest of the day, the lights were programed to simulate the sunshift and daily variation in the brightness of the sky in accordance with the direction of the magnetic field (either normal or reversed). Lights, heaters, and coils were d.c. powered from distant voltage and current sources. The wiring was tightly twisted and judiciously installed to keep unwanted electric and magnetic fields from the animals' habitat. Extraneous noise levels were low at our test site in the undisturbed woods of the Quissett campus.
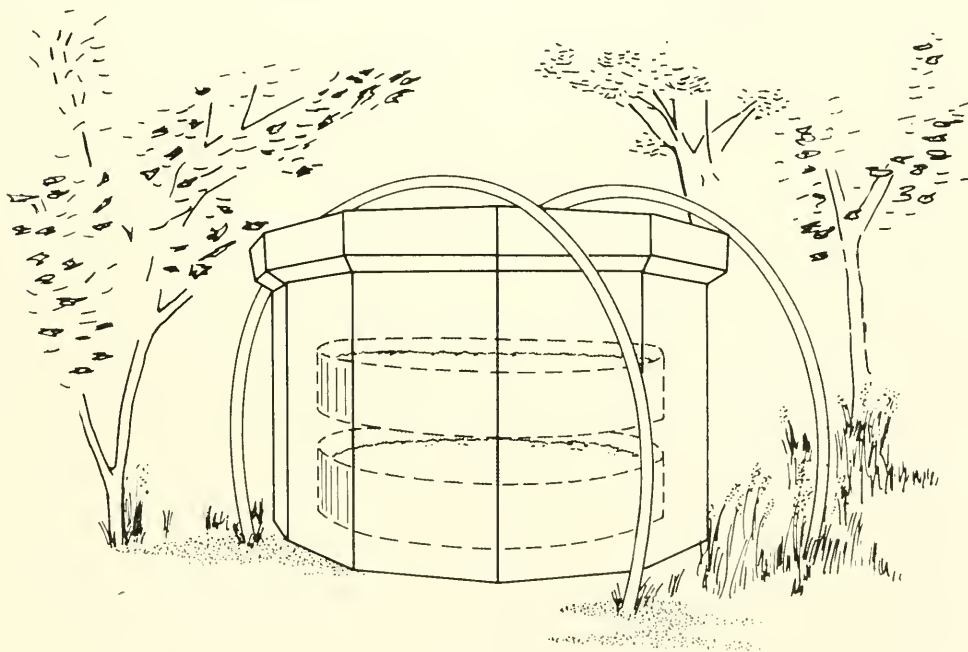
Figure 9   Magnetic test facility on the Quissett campus of the Woods Hold Oceanographic Institution. The two large, north-south oriented Helmholtz coils control the horizontal component of the magnetic field in the hut. The actual training experiments are conducted in the top tank. The lower pool holds extra stingrays in reserve. (After Kalmijn 1977b.)

Each morning, two observers conducted a series of 10 to 20 trials after first turning on the lights, shutting off the flow of air, and checking the direction of the magnetic field. Then they simultaneously introduced two circular enclosures into the pool, one in the magnetic East, the other in the magnetic West (Figure 10). The enclosures consisted of plastic tubs, 30 cm in diameter, with a 20-cm-wide opening near the bottom for the stingrays to enter. After positioning the enclosures at a distance of 25 cm from and with the openings toward the wall of the pool, the observers stepped back to watch the behavior of the animals from behind a black felt screen. When one of the stingrays entered either the east or west enclosure, both observers blocked off the opening by lowering a gate. If the animal chose the east, by definition the correct enclosure, it was rewarded with a small piece of herring; if the animal took the west or incorrect enclosure, it was gently prodded with a blunt Plexiglas rod as a form of punishment. Eventually, the enclosures were moved to the magnetic North and South of the pool, and the animal was set free for the next trial, to start 1 to 2 min later.

It took the animals some time before they learned to rely on the magnetic field in deciding which side of the pool to avoid and where to go for food. Once conditioned, however, two of the stingrays made the highly significant scores of 56 and 164 correct vs 22 and 84 incorrect choices respectively ($P <$ 0.001, calculated with the chi-square, corrected for continuity). A third ray had to be removed because it could not compete and was actually losing weight. After the initial training, the stingrays' orientational performances
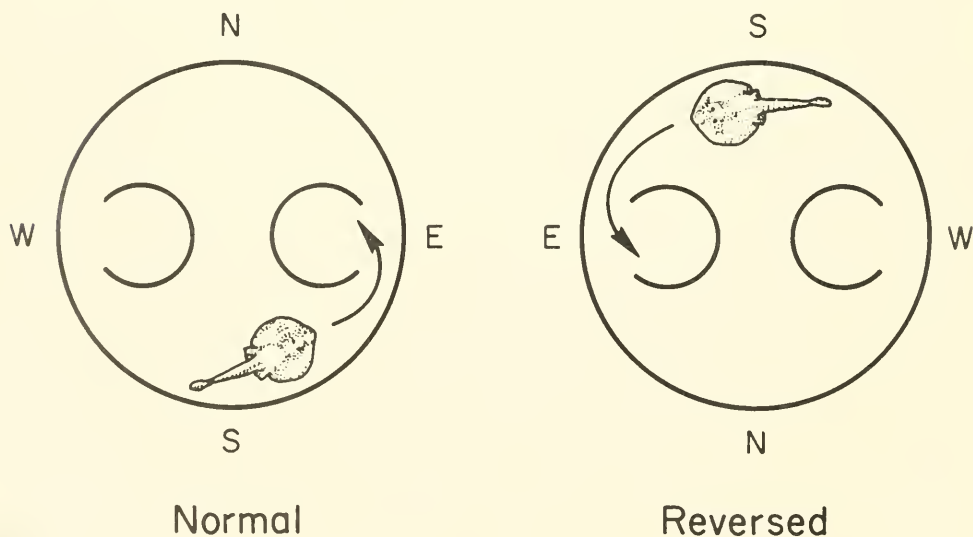
Figure 10 To receive reward and to avoid punishment, the stingray *Urolophus halleri* ignores the enclosure in the magnetic west to enter the one in the magnetic east. The magnetic nature of the animal's response becomes evident upon reversal of the field: the stingray again enters the enclosure in the magnetic east, though it is now located at the opposite side of the pool. (From Kalmijn 1977*b*.)

did not significantly change during the individual sessions or from day to day. This, we felt, allowed us to treat the trials as independent choices, despite the facts that the field was reversed only once a day and in systematic order, that is, each night before the next morning's series of 10 to 20 trials. In a follow-up series, we changed the direction of the field again on a daily basis, but this time in random order. Under this regime, our most active stingray made, without further training, 120 correct vs 64 incorrect choices in a period of 15 days ($P < 0.001$).

In earlier tests, frequent field reversals appeared to confuse the animals. Yet, after refining both the setup and experimental procedure and progressively gaining more experience, we recently began a series in which the direction of the magnetic field was altered randomly from trial to trial to provide the strongest evidence possible. In these most crucial experiments, the field was set by a third person, while the two observers did not know whether to feed or punish until after the stingrays had made their choice. The change of the field was made during the commotion of feeding or punishment by first slowly turning the coil current down to zero and then up again in either the normal or reversed direction. After being released at the north or south of the pool, the animals usually swam about briefly, often stopping at the entrance of both the east and west enclosures before making their final decision. Under these double-blind conditions, our fastest performing ray at times attained scores as high as 9 out of 10, and upon completion of the series totaled 101 correct vs 53 incorrect choices, which again is significant at the extremely conservative level of $P < 0.001$.

Since we know that marine elasmobranchs, when cruising through the earth's magnetic field, inevitably induce electric fields that (1) are well within the sensitivity and frequency range of their acute electric sense and (2) strictly correlate with the magnetic compass direction in which the fishes are heading, it appears reasonable to assume that the stingrays' geomagnetic orientation reflects the animals' proposed electromagnetic sensory abilities. Granted this inference to be correct, the ampullae of Lorenzini undoubtedly are the pertinent receptors. They not only are the sense organs that render sharks, skates, and rays sensitive to electric fields, but they also have the proper spatial arrangement and physical disposition for the reception of the induced electromagnetic voltage gradients (Kalmijn 1973, 1974). As yet, attempts to electrophysiologically demonstrate the receptors' sensitivity to motion through the earth's magnetic field have suffered from a misconception of the physics involved (Andrianov et al. 1974; Akoev et al. 1976). A change of magnetic flux imposed on a stationary animal in a stationary environment is principally different from a change of flux resulting from an animal's motion with respect to its environment in the presence of a constant magnetic field. Nevertheless, a preview of unpublished data indicates that conclusive evidence is to be expected soon (Brown and Ilyinsky 1977).

In the ocean, electric fields of geophysical and geochemical origin may also play an important role in the elasmobranchs' life. Thus, in addition to the magnetic experiments, we are training the stingray *Urolophus halleri* to

orient with respect to uniform electric fields such as those induced by ocean currents flowing through the earth's magnetic field (passive electro-orientation). These fields typically measure from 0.05 to 0.5 μV/cm (Von Arx 1962). At the present writing, the stingrays have learned to orient perfectly well in uniform d.c. fields as low as 0.04 μV/cm, which proves their ability to detect the direction and polarity of the oceanic electric fields. During these tests, the horizontal component of the earth's local magnetic field is nulled, whereas the vertical component is left undisturbed. By gradually lowering the electrical field strength, we will establish the threshold of response. Yet, the significance of many other natural and man-made voltage gradients in the ocean remains to be evaluated. Obviously, we still are at a pioneering stage and a long way from a full understanding of the electric and magnetic sensory biology of sharks, skates, and rays.

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VI ECOLOGY AND BEHAVIOR

DISPERSION OF THE PORT JACKSON SHARK

IN AUSTRALIAN WATERS

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Much of the information on shark populations is derived from catch records, mainly of commercial species outside Australian waters (Ford 1921, Hickling 1930, Templeman 1954, Strasburg 1958, Springer 1960, 1963, 1967, Holden 1965, Bullis 1967, Davies and Joubert 1967, Kato and Carvallo 1967) but little is known of natural shark populations in Australian waters (Olsen 1954, McLaughlin and O'Gower 1971). While many interesting studies of shark behavior have been made using scuba diving equipment (Eibl-Eibesfeldt 1965, Limbaugh 1963, Nelson 1969, Johnson and Nelson 1973, and others) few attempts have been made to apply the advantages of using scuba equipment in the organized study of natural, field populations of sharks (Nelson and Johnson 1970, McLaughlin and O'Gower 1971). These latter, detailed, underwater studies on heterodontids have added greatly to our understanding of the activities of these sharks, especially *Heterodontus portusjacksoni* in Australian waters. Together with the studies reported here, they give a very complete picture of the ecology of this shark.

Tagging of *H. portusjacksoni* (McLaughlin and O'Gower 1970) in conjunction with studies on shark movements (McLaughlin and O'Gower 1971) or on shark populations (Nash and O'Gower, unpublished data) has yielded much information on the dispersal behavior of *H. portusjacksoni*, both on inshore reefs and along Australia's eastern seabord.

INSHORE POPULATIONS

Aggregates of *H. portusjacksoni* have been observed by the authors and others, mainly during August and September, as far north as Yagen (near Seal Rocks) and Port Stephens, and as far south as Jervis Bay. In the Sydney area aggregates have been sampled at 25 generalized localities, with specific caves and trenches used year after year (Figure 1), but the most detailed study has been of the populations frequenting the South Bondi reef (Figure 2). McLaughlin and O'Gower studied these from 1962 to 1964.

During the 95 surveys of the South Bondi reef over the 3-year study period, the numbers of *H. portusjacksoni* seen were correlated inversely with water temperature and directly with the reproductive cycle. Numbers were generally low from November to June, with an early, seasonal peak in July or early August, followed by a drop and a subsequent rise to a maximum peak of gravid females in September to early October (Figure 3). Of the sharks seen on the reef 85% were females, the bulk of which were sighted in October, whereas of the sharks caught on long lines in deep water by fishermen 58% were females. It may therefore be concluded that the mature males in the population spend most of their time in deeper water, only moving inshore in small numbers from March to July. On the South Bondi reef, horn sharks were sighted at only six locations in spite of frequent, widespread surveys (a-f in Figure 2). A general preference for sites a and b was observed, the other sites being used mainly during the breeding season to accommodate the large influx of visitors. Site e, a very large cave close to shore, on the
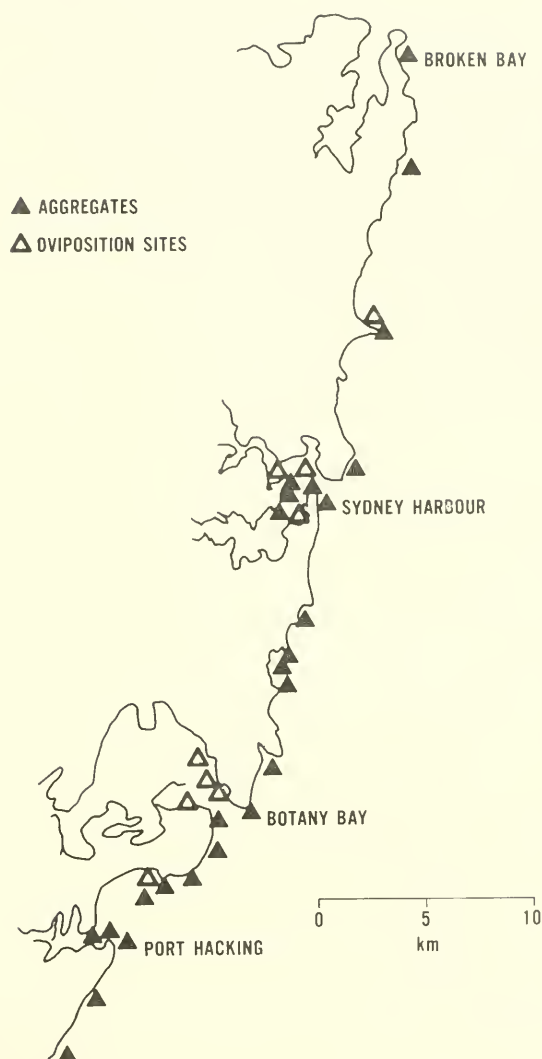
Figure 1   Location of aggregates and oviposition
sites for *H. portusjacksoni* in the Sydney area.

other hand sheltered large numbers of reproductively spent females for very
brief periods near the end of the season. It is possible that this cave served pri-
marily as a traditional place where postbreeding females gathered before
their southward migration.

Over the 3-year period 665 shark sightings were made on the South Bondi
reef, of which 83 were made before the tagging program; 158 were tagged
and 146 were resighted, giving a minimum of 30% tagged sightings, and an
estimated population well in excess of 200 sharks. The longest recorded in-
terval between tagging and resighting was 6 years, though most recoveries
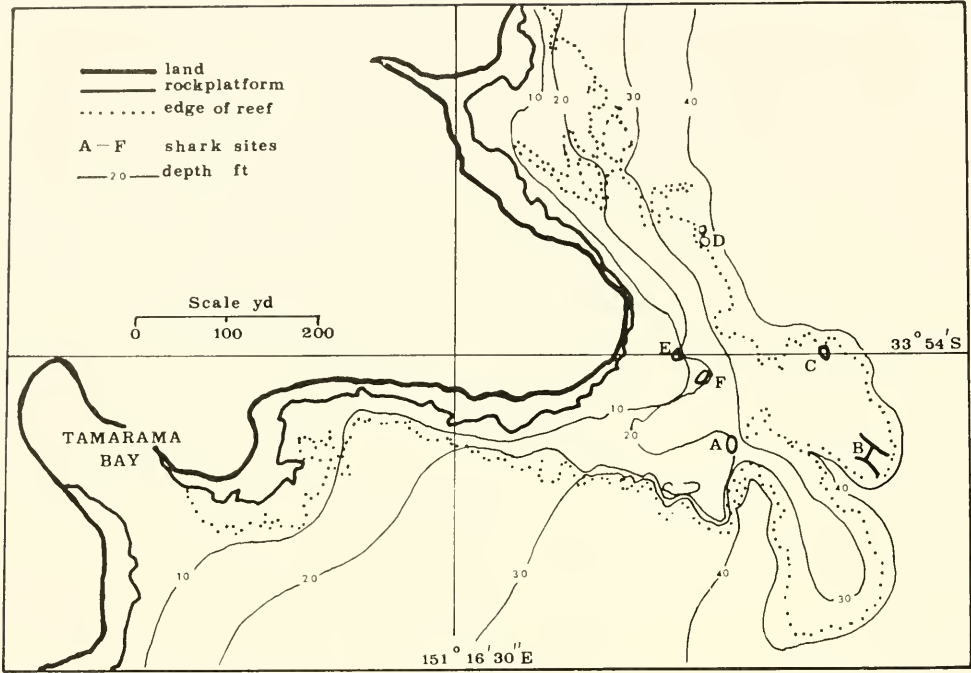
Figure 2   Resting sites for *H. portusjacksoni* and depth contours on shallow reef at Bondi, Sydney.
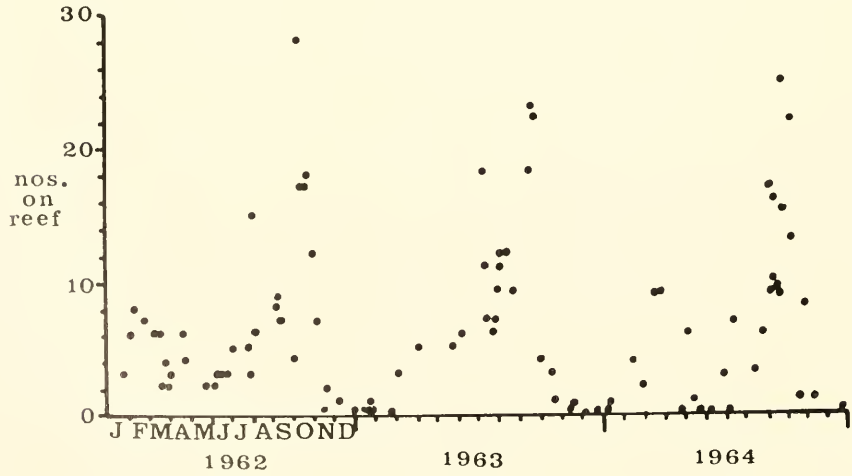
Figure 3   Seasonal changes in the number of *H. portusjacksoni* on the reef at Bondi.

were made within a month after tagging. Almost twice as many resightings were made after an interval of 4 months than were made over the period of 1 to 4 months. In other words, most sharks were resighted either within a week of tagging or 4 months afterwards. In more recent studies (Nash and O'Gower, unpublished data) several sharks transferred from particular reefs and bays in the Harbour (Rose Bay and Middle Head) have been recovered at their original resting site at the Sow and Pigs, while some natural traffic has been noted between various reefs in the Harbour (e.g., from South Head to Middle Head and Sow and Pigs, and from Sow and Pigs to Middle Head). McLaughlin and O'Gower (1971) also recorded one horn shark that moved from Middle Head to the South Bondi reef, while a shark tagged at Jervis Bay was found in nearby Disaster Bay and later relocated at the tagging site in Jervis Bay (Table 1).

These changes in numbers and patterns of shark movements on inshore reefs indicate that most of the sharks were continually moving from and

Table 1. Tagging and recapture data for *H. portusjacksoni*

	Tagging data			Recapture locality	Distance traveled (km)	Depth recapture (m)
No.	Date	Locality	Date			
1	24.9.70	Sydney	14.4.71	40 km S.E. of Wilson's Promontory	760	?
2	15.10.70	Sydney	25.11.70	Ulladulla	200	?
3	25.20.70	Jervis Bay	8.6.72	Disaster Bay	10	8
			26.8.72	Jervis Bay	—	5
4	4.9.71	Jervis Bay	28.11.71	Cape Everard	400	60
5	4.9.71	Jervis Bay	28.3.72	27 km E. Port Arthur	720	40
6	4.9.71	Jervis Bay	2.5.72	37 km E. Lakes Entrance	600	40
7	4.9.71	Jervis Bay	10.12.71	Ulladulla	40	?
8	11.9.71	Jervis Bay	Jan. 73	Port Albert	620	"shallow"
9	5.10.71	Sydney	6.11.71	Wreck Bay	130	"shallow"
10	5.10.71	Sydney	1.2.73	Island Point	800	12
11	1.5.72	Sydney	28.7.73	Deal Island	750	70
12	6.1.72	Sydney	24.3.73	30 km S.E. of Barracouta Oil Rig	750	?
13	3.9.72	Sydney	14.10.72	Kiama	110	5
14	7.9.72	Sydney	30.6.74	3 km E. of Cape Naturaliste	850	230

returning to reefs after short absences, and many of these absences could be related to oviposition. The return of sharks to the same small sites on reefs, after natural and artificial displacements of up to 6 years, and their movements between specific localities on the same reef, indicate clearly that *H. portusjacksoni* has a precise and well-developed homing ability.

When the female *H. portjacksoni* visit inshore reefs during September and October to lay eggs, the oviposition sites are invariably in sheltered harbours, bays, and inlets (e.g., Sydney Harbour, Botany Bay, and Jervis Bay), and eggs are deposited on the sheltered areas of a very few inshore reefs (e.g., Long Reef). Eggs are found either in crevices or lying on the open sand and at depths ranging from 1 to 20 m, the vast majority being in the depth range of 1 to 5 m. These eggs can be classified according to age and to contents. The presence of mucous indicates an age of a few weeks, a flexible shell case means that the eggs are a few months old; old eggs are identified by a rigid shell case with spirorbid worms or a brittle shell case containing a fully developed embryo. The contents of the egg case are stated simply as empty or full. In Jervis Bay, 149 eggs were collected in rock crevices and on the sand, with the result shown in Table 2. If these data are analysed in 2 × 2 contingency tables for habitat × contents, the chi-square values and probabilities are as follows:

Old eggs $\chi^2 = 3.355$ 1 df
            $0.10 > P\ 0.05$
New eggs $\chi^2 = 18.505$ 1 df
            $P < 0.01$

Obviously the association between egg deposition site and egg case contents is not random. The data show that (a) eggs are preferentially deposited in rocky crevices, (b) rock crevices are at a premium, and (c) eggs deposited in crevices have a significantly higher probability of survival than eggs deposited on the sand. (As most empty egg cases seen were ruptured rather than drilled, as female sharks have been seen carrying eggs in their mouths presumably

Table 2. Record of eggs found in Jervis Bay.

| Habitat | Age and contents | | | |
| | Old | | New | |
	Empty	Full	Empty	Full
Crevice	4	30	0	46
Sand	4	0	55	0

to deposit them in rock crevices, and as male sharks have been observed to spit out crushed eggs presumably having swallowed their contents, it seems that egg mortality is mainly the result of adult shark predation, either accidental or deliberate, rather than drilling by carnivorous gastropods (Grover 1972).)

Several localities in sheltered bays and inlets appear to be traditional oviposition sites, as eggs have been seen in particular crevices over several years. Fresh eggs may be deposited over old eggs containing fully developed embryos, with empty, brittle egg cases deeper in the crevice. The location of such sites in sheltered bays and inlets is fortuitous and selective in that the juveniles develop and grow in the estuaries.

MIGRATORY BEHAVIOUR

Almost all shark species studied have been found to migrate seasonally (Olsen 1954, Kauffman 1950, Backus et al. 1956, Holland 1957, Strasburg 1958, Springer 1960, 1967, Holden 1967). McLaughlin and O'Gower (1971), from their limited tag returns (four recaptures, or 1.5% recapture rate), postulated a north-south axis for the migratory route of the *H. portusjacksoni* on the eastern seabord of Australia.

Continued underwater studies by the authors on *H. portusjacksoni* has given a 6% recapture rate, with 14 tags recovered from 230 sharks tagged. This rate compares favourably with other recapture rates for sharks. Olsen (1954) reported a 4% tag recovery for *Galeorhinus australis*, and Holden (1967) and Aasen (1960) recorded 5% and 6% recoveries respectively in their studies of *Squalus acanthias*. As the probability of recapturing a tagged shark is obviously much greater for commercial species than for harmless, noncommercial species such as *H. portusjacksoni*, our 6% return was commendable.

The sites of tagging and recapture for the 14 recently recovered tags, plus McLaughlin and O'Gower's (1971) four previous tags, are shown in Figure 4. Three animals (1, 2, and 14) were recaptured over 700 km from the tagging site, the longest journey being about 850 km. The highest minimum rate of movement away from the tagging site was achieved by shark no. 9, which covered approximately 200 km in 31 days, a speed of 6.5 km/day. This rate greatly exceeds that recorded by McLaughlin and O'Gower (1971) with shark 16, which was caught 223 days after tagging and some 400 km from the point of tagging, a minimum rate of 1.8 km per day. Sharks 2, 9, 13, and 18 were captured in shallow water close to the coast. Sharks 4, 5, 6, 11, 12, 15, and 16, on the other hand, were captured in deep water during the summer and autumn.

From the data in Table 1 it appears that the sharks migrate southward in early Australian summer, staying close inshore. They then spend the summer and autumn months in the deep waters of Bass Strait, and begin their northward migration in winter. The evidence from our studies, discussions with professional fishermen, and personal experience on trawlers indicates that this northward movement occurs in the deeper offshore
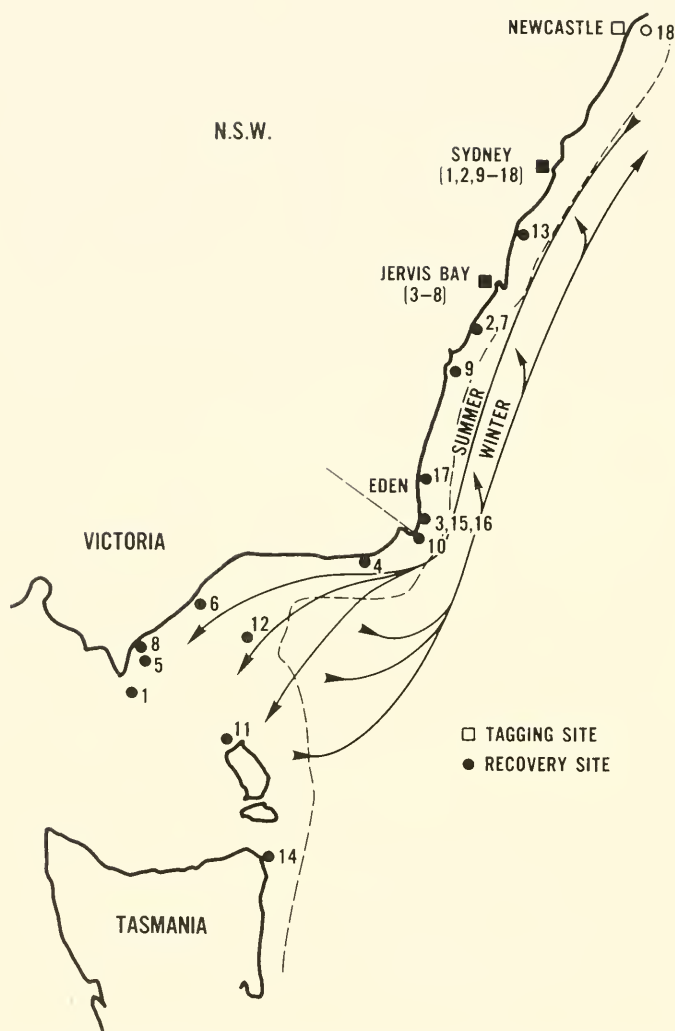
Figure 4   Tagging and recapture sites and proposed migratory routes of *H. portusjacksoni*.

waters, for the sharks are rarely caught by longline, and few are seen with scuba, in waters less than 60 m or closer than about 25 km from the coast before the August/September influx of female sharks towards the coastal breeding grounds (McLaughlin and O'Gower 1971). The proposed migratory route for *H. portusjacksoni* is given in Figure 4; this route is not inconsistent with the movement of the warm and cold water masses along the eastern seabord and Bass Strait (Rochford 1974), especially if Springer's (1963) correlations between shark movements and water temperature apply to *H. portusjacksoni*.

POPULATION STRUCTURE

Since *H. portusjacksoni* have such a wide distribution (from Western Australia to beyond Newcastle in central N.S.W., with a doubtful record in Moreton Bay in Queensland (Saville-Kent 1897)), and since the apparent major oviposition and nursery areas are extensive but widely separated (e.g., Port Stephens, Sydney area, and Jervis Bay area on the N.S.W. coast), it would seem reasonable to expect that different populations of sharks could use different breeding areas and hence be genetically different. Any method by which genetic variation between individuals of a species can be detected should yield information about the existence of separate populations within a species. McLaughlin and O'Gower were unable to distinguish morphologically between individuals of *H. portusjacksoni*, but Gordon (1947), using the frequencies of genes specifying morphological characters in the fish *Platypoecilus maculatis*, was the first to demonstrate the existence of subpopulations in a marine vertebrate. Techniques and methodology have advanced considerably since that time, and population studies today usually involve genetic differences measurable at the molecular level. Cushing (1964) reviewed the results of such studies involving marine vertebrates, but his survey covered mainly serological techniques, high-resolution electrophoresis of proteins being then in its infancy. In a later, very extensive review, de Ligney (1969) surveyed the principles, methods, and results of virtually all serological and biochemical studies of fish populations undertaken to that time. This study reports the first attempt to define the population structure of a species of elasmobranch by biochemical means, although Sindermann and Mairs (1961) have described an erythrocyte antigen system in *Squalus acanthias* and proposed that it be used in population studies of this shark.

A typical cellulose acetate gel, on which several shark haemolysates have been separated, is shown in Figure 5. The proteins running between the origin and the haemoglobins are polymorphic (Nash and O'Gower, unpublished data). The simplest genetic explanation for the protein variation assumes a triple, codominant allelic system (a, b, and c) that segregates according to $(p + q + r)^2$, where p, q, and r represent the gene frequencies. From Table 3 it can be seen that the populations from Sydney, Jervis Bay, Victoria, and South Australia are in good agreement with the Hardy-Weinberg law, the Western Australia population agrees at the 5% level, and the Newcastle population differs significantly from the law.

Comparisons of the gene frequencies show that superficially the populations are all different. However, the statistically more rigorous χ^2 pairwise comparisons (Table 3) do not substantiate this belief. While there are small, but significant, differences among the three eastern populations, and among the Sydney, South Australia, and Western Australia populations, there is no significant difference (5% level) between the Jervis Bay and the remaining southern and western populations.

The results of the present study are probably as good as one could expect from a survey of natural populations, particularly when it has proved
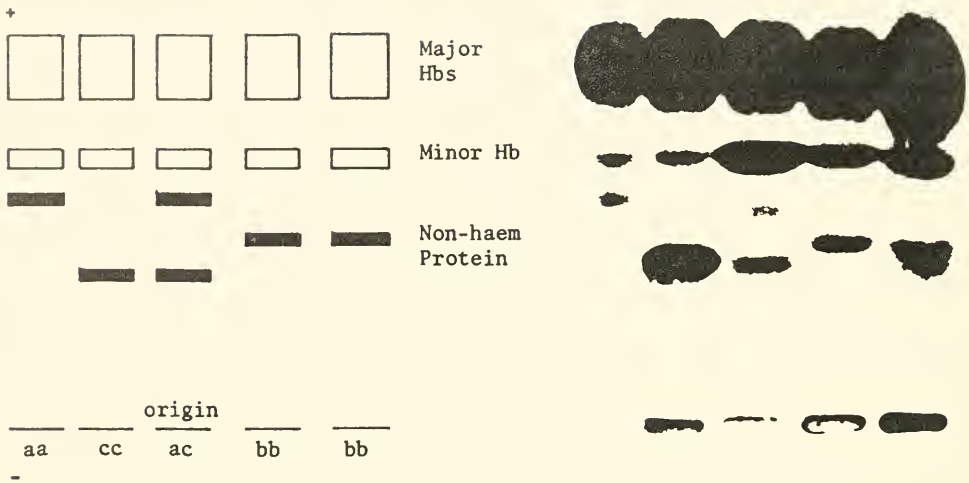
Figure 5 Hemolysates from individual sharks were separated on cellulose acetate gels by ionophoresis at pH 8.6. The polymorphism of the non-haem protein was detected by staining the gel with 0.2% Amido Black. On the gel shown there are four of the six possible phenotypes.

Table 3.   Gene frequencies, observed and expected phenotypes, chi-square and probabilities for haemolysates for various populations of *H. portusjacksoni.*

Locality	O/E	Phenotypes						Gene frequencies			χ^2	P
		aa	ab	ac	bb	bc	cc	p	q	r		
Newcastle	Obs.	2	24	6	6	5	1	0.386	0.466	0.148	9.0	<0.05
	Exp.	6.5	15.5	5	9.5	6.1	1					
Sydney	Obs.	2	13	11	36	42	10	0.123	0.557	0.320	1.23	<0.8
	Exp.	1.7	15.6	9	35.3	40.6	11.7					
Jervis Bay	Obs.	6	14	17	16	31	15	0.217	0.388	0.394	0.97	<0.9
	Exp.	4.6	16.7	17	14.9	30.3	15.4					
Victoria	Obs.	0	2	4	5	4	3	0.166	0.444	0.389	3.3	<0.5
	Exp.	0.5	2.6	2.3	3.5	6.2	2.7					
S. Australia	Obs.	6	4	6	5	6	3	0.366	0.333	0.300	3.4	<0.5
	Exp.	4	7.3	6.6	3.3	6	2.7					
W. Australia	Obs.	3	3	13	6	8	12	0.244	0.255	0.500	6.3	<0.1
	Exp.	2.7	6	11	2.9	11.5	11.2					

so difficult to obtain adequate numbers of animals for all but two of the populations. It must also be considered that only the Sydney and Jervis Bay populations were sampled inshore and on the breeding grounds; hence, it is possible that the animals taken in the other areas are not all members of the same subpopulation. However, in general the results illustrate well some of the basic principles of population genetics. They also lend some support to the theory of the presence of reproductively semi-isolated

subpopulations among the sharks. (Semi-isolated subpopulations may be defined, for the purposes of this discussion, as adjacent groups of animals of one species between which there is limited genetic interchange.)

From the data in Tables 3 and 4, it appears that the species is divided into at least two major populations. The eastern population extends from the northern limits of the sharks' range to the vicinity of Jervis Bay, and the western population probably extends southward and eastward from the northeastern Victorian water. However, while the Jervis Bay population does not differ significantly from the more western populations it is significantly different from the Sydney population, and the Newcastle population differs significantly from all other populations. (For reasons discussed more fully later, the latter deviation may be due to other factors.) Consequently, the eastern population could be comprised of two or three subpopulations based on the Newcastle, Sydney, and Jervis Bay breeding grounds.

The existence of two major populations is in accord with the patterns of flow of the principal ocean currents around the southern half of the continent (Figure 6). These currents could direct the movements of the sharks at migration time and hence act as a natural barrier between the two populations. However, the current patterns of Figure 6 apply to the major currents off the Continental Shelf, which is beyond the shark's range. While little is

Table 4. Chi-square values and probabilities for pairwise comparisons of gene frequencies between populations of *H. portusjacksoni* from various localities.

Localities	χ^2	P
Newcastle/Sydney	39.5	<0.001*
Newcastle/Jervis Bay	28.5	<0.001*
Newcastle/Victoria	13.8	<0.05*
Newcastle/S. Australia	15.5	<0.01*
Newcastle/W. Australia	29.1	<0.001*
Sydney/Jervis Bay	12.8	<0.05*
Sydney/Victoria	4.4	0.50
Sydney/S. Australia	19.9	<0.01*
Sydney/W. Australia	26.4	<0.001*
Jervis Bay/Victoria	2.95	<0.8
Jervis Bay/S. Australia	6.4	<0.3
Jervis Bay/W. Australia	7.99	<0.2
Victoria/S. Australia	4.3	0.5
Victoria/W. Australia	3.2	<0.5
S. Aust/W. Australia	7.3	<0.2

*Significant difference in gene frequencies.
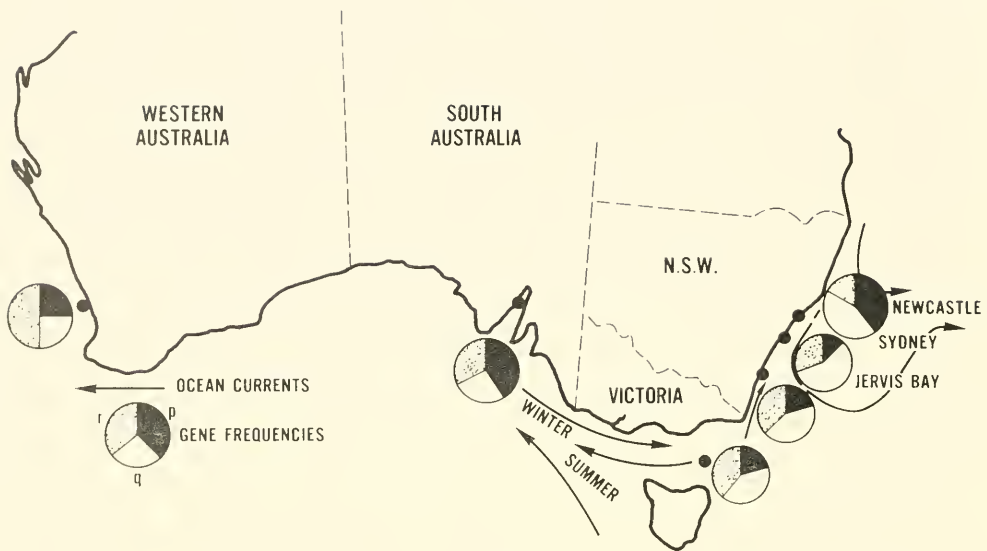
Figure 6   Ocean current systems and gene frequencies of *H. portusjacksoni* from various places off the southern shores of Australia.

known of the inshore current patterns, Rochford (1974) states that inshore countercurrents accompany the major currents off the Continental Shelf and that these countercurrents could act as the barrier between the two shark populations. The migratory movements of *H. portusjacksoni* do indicate the presence of some natural barrier across Bass Strait (See "Migratory Behaviour").

McLaughlin and O'Gower (1971) have shown that the sharks return to the same area year after year to breed. These studies, plus the present study, have delineated two major breeding sites on the east coast, at Sydney and Jervis Bay, and have failed to find any significant breeding site between these two localities. Although Sydney and Jervis Bay are only about 140 km apart, the differences in phenotypic distribution between the two populations taken from these areas (Table 3) are significant (Table 4). Finally, sharks tagged in either the Sydney or the Jervis Bay areas have not been recorded in the other area at breeding times. From these arguments it seems reasonable to postulate that these two subpopulations of *H. portusjacksoni* can be termed semi-isolated subpopulations.

The distribution of phenotypes in the two major populations sampled, Sydney and Jervis Bay, are in such good agreement with the Hardy-Weinberg Law that the genetic hypothesis of three codominant alleles coding for the polymorphis protein is, theoretically, fully substantiated. Practically, however, in all such studies, "where pedigree data can be obtained from laboratory breeding, the genetic basis of the variation can be unequivocally determined" (Koehn 1972). Although the Port Jackson shark will breed readily in captivity, there were (and are) no facilities available to the authors for testing the genetic hypothesis in this manner.

The deviation from Hardy-Weinberg equilibrium of the Newcastle population, and the poor agreement of the Western Australian population, could be due to the smaller sample size or to sampling error (Wright 1964), but other factors cannot be ruled out. The Western Australian population consisted largely of juvenile animals, while about 30% of the Newcastle sample were juveniles. Hashimoto and Matsuura (1960) and Vanstone et al. (1964) have described developmental changes in the concentration and number of types of haemoglobins in *Oncorhynchus* sp. Manwell (1963) showed that foetal and adult shark haemoglobin differed in primary structure. It is conceivable, then, that phenotypic patterns in juvenile and adult *H. portusjacksoni* are sufficiently different to admit the possibility of miscounting. This could explain the discrepancies in the two populations.

A further possibility, in the case of the Western Australian population, is that the animals sampled were not members of a single breeding population, but a random sample drawn from several subpopulations. Under these conditions, and invoking Wahlund's Principle (Li 1955), there would be an apparent shortage of heterozygotes and an excess of homozygotes in the sample. These discrepancies are apparent in Table 3. As the sharks in the Western Australian population were captured several kilometers from shore, it is entirely possible that they were from the general population of the areas, and not from a specific breeding site.

Unfortunately, the distribution of phenotypes in the Newcastle population cannot be explained in this manner. Possibly the genetic hypothesis, obviously adequate for the other populations, is untenable in northern waters. Newcastle is quite close to the northern limits of the range of *H. portusjacksoni* on the east coast and, although the identity and function of the polymorphic protein is unknown, selection against the homozygous condition may be occurring.

Returning to the distribution of gene frequencies (Table 3), it is obvious that there is considerable variation among populations. The reasons for the differences are not so obvious. Lewontin (1974) stated that, "spatial variation in allelic frequencies, unlike temporal changes, cannot provide any information on the intensity of selection, because, in the absence of historical evidence, it must be assumed that the spatial pattern is an equilibrium state. . . ." Selection can of course be detected if there is a geographical cline in the gene frequencies of several spatially separated populations. Thus Sick (1961, 1965) and O'Gower and Nicol (1968) were able to demonstrate a latitudinal cline in the frequencies of the genes coding for haemoglobin in *Gaddus* sp. and *Anadara* sp. respectively. In the latter case the environmental variable responsible for the alteration in gene frequencies has been identified (Nicol, Collette and O'Gower, unpublished data). Detecting temporal changes in gene frequencies requires data from at least two generations. Considering the habitat, behaviour, and generation time (approximately 10 to 12 years) of *H. portusjacksoni*, such data are virtually impossible to obtain.

One must therefore use the data available. These data (Tables 3 and 4) indicate no regular pattern, but rather a mosaic of gene frequencies. Kimura and Weiss (1964) have shown mathematically that in the absence

of selection such a patchy distribution of gene frequencies can occur by random drift of neutral mutations, with some migration between adjacent populations and an occasional, distant migration. Experimental verification of this theory came from the work of Selander (1970), on whose results Lewontin (1974) commented, "It would be absurd to imagine we are observing selection gradients. For this reason we cannot deduce selection from patchy and mosaic gene frequency distributions." On the other hand, Wallace (1968) and Lewontin (1974) have derived equations showing that in two populations of 10 000 individuals each, the exchange of 10 individuals per generation is, in the absence of selection, sufficient to bring the gene frequencies in the two populations to a common value.

In the case of the two shark populations using the Sydney and Jervis Bay breeding sites, the differences in gene frequencies could be due to random genetic drift, for the distribution of gene frequencies appears analogous to those described by Selander (1970). However, the migration of this shark between the two localities (McLaughlin and O'Gower 1971, and present authors) would seem to indicate that differences in gene frequencies could be eliminated, unless the populations using the two breeding sites are temporally isolated. The evidence to solve this possible contradiction is not available. Interpretation of the data and resolution of the problem depend entirely on which school of population genetics one follows, the classical (Clarke 1975) or the non-Darwinian (Kimura 1975). The conflicting approaches of the two schools are evident in the lack of a definitive answer to the problem discussed herein. The first prerequisite for resolving the issue is that the function of the polymorphic protein be determined, although even with this knowledge the detection of genetic selection would be an enormous task. The second is that more and larger samples be obtained, during a single season, from breeding populations which are inshore. From the present study one can conclude that within the two major populations there is some division into smaller breeding units, but they are not reproductively isolated. One can only conclude, in the absence of evidence to the contrary, that the observed differences are the result of random genetic drift.

It therefore appears that further studies on the ecology of *H. portusjacksoni* do not warrant the effort involved. Nevertheless, even with the very limited resources available, the application of scuba diving equipment to the study of sharks has resulted in a considerable addition to our knowledge of sharks in their own environment.

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PROBLEMS IN STUDIES OF SHARKS IN THE SOUTHWEST INDIAN OCEAN

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Courtesy of *The Daily News*, Durban, South Africa

INTRODUCTION

The background data for the ideas presented here are taken chiefly from the results of a taxonomic and biological survey of the sharks of the east coast of southern Africa undertaken by the Oceanographic Research Institute in Durban (D'Aubrey 1964; Bass et al. 1973. 1975*a*, 1975*b*, 1975*c*, 1975*d*, 1976). This survey was begun in 1959 by Jeanette D'Aubrey under the guidance of the late David Davies and continued by the author from early 1968. Continuity was ensured by the expert technical assistance of Nadraj Kistnasamy, who also provided many of the original observations.

The scientific names used here follow those used in the final results of this survey. It should be noted that the shark described under the name *Carcharhinus spallanzani* will soon be described as a new species by Dr. J. A. F. Garrick; the specific name *spallanzani* is incorrect.

PHYSICAL ENVIRONMENT OF THE SOUTHEAST AFRICAN COAST

The east coast of southern Africa, arbitrarily defined here as ranging from Beira in the northeast to Knysna in the southwest, is shown in Figure 1 together with the major current systems of the southwestern Indian Ocean. The temperature regime of the waters bathing the east coast varies from tropical in the north to warm temperate in the south. In the northeast, warm water is supplied by the South Equatorial Current driven from the central Indian Ocean towards the African coast by the southeast trade winds. In the southwest part of the region is cool Atlantic water from the Benguela Current, which flows northward along the west coast of southern Africa. Further to the south, at a latitude of about $42°$, lies the subtropical convergence and the cold West Wind Drift.

The South Equatorial Current divides near Madagascar, one stream flowing westward to strike the African coast slightly south of the equator, the other flowing along the east and south coast of Madagascar and eventually reaching the African coast at about latitude $25° $ S to flow southwards as the Agulhas Current. This follows the edge of the relatively narrow continental shelf, indicated in Figure 1 by the 100-fathom depth contour. At about $34°$S the current is deflected from the coast by the extensive area of continental shelf known as the Agulhas Bank and eventually turns southward and eastward to become the Return Agulhas Current. The surface currents to the south of the Agulhas Bank are confused by interactions between the Agulhas and Benguela currents, while the Agulhas Bank is covered by a mixture of warm Agulhas and cool Atlantic water.

The flow of the Agulhas Current varies according to season. During the southern summer the Somali Current of the northeast African coast flows southward under the influence of the northeast monsoon and joins part of the South Equatorial Current to form the Mozambique Current, which in turn merges with the Agulhas Current. During the southern winter the Somali Current flows northward because of the southwest monsoon and the
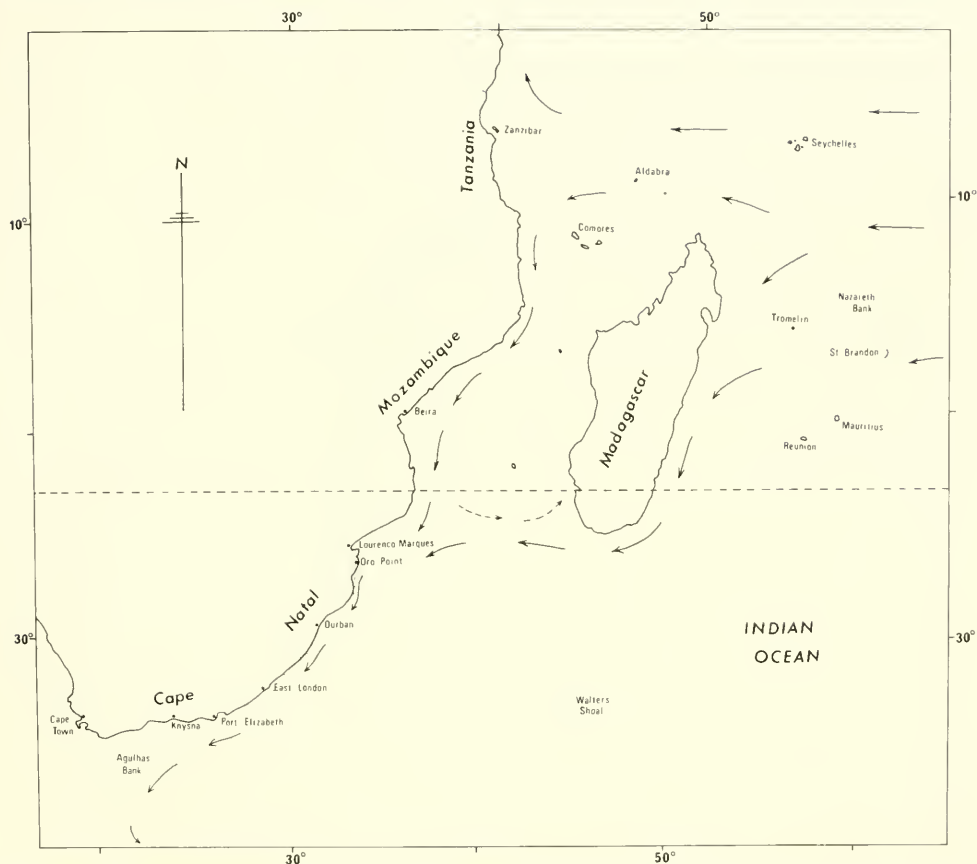
Figure 1   The southwest Indian Ocean with an indication of the major current systems. The 100-fathom depth contour is shown as a dotted line from Beira southwards along the African coast. (After Figure 1 of Bass et al. 1973.)

Mozambique Current is thus reduced in strength. In addition, the latter may form a discrete circulation in the Mozambique channel at this season, so that the Agulhas Current is fed by only that part of the Equatorial Current that runs along the east coast of Madagascar. The Agulhas Current thus penetrates furthest along the east coast during summer, when warm Agulhas water may extend as far as the Cape Peninsula. In winter the relatively cool south coast waters may extend as far north as Natal in the form of an inshore counter-current.

The distribution of pelagic animals along the east coast thus depends on the season, while that of sedentary animals is limited by the extremes of temperature to which they may be subjected at different times. Seasonal changes of inshore surface temperatures at four localities on the southern African coast are shown in Table 1.

Table 1.   Average monthly surface temperatures

Locality	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
						(nearest °C)						
Oro Point	26	26	26	25	24	23	22	21	22	22	24	25
Durban	24	24	24	23	22	19	19	19	20	21	22	23
Port Elizabeth	22	22	21	18	17	16	16	16	16	18	18	20
False Bay	18	18	16	16	14	14	13	13	13	14	15	17

Upwellings of cold subsurface water are not uncommon along the eastern and southern Cape coasts, so inhabitants must withstand sudden changes of water temperature (of the order of several degrees Centigrade) as well as the more gradual changes taking place with the passing of the seasons. This may well be the chief reason for the high degree of endemism among the fauna and flora of this region.

The inshore currents of the east coast are mainly eddies of the Mozambique and Agulhas currents and are most pronounced where the continental shelf is relatively wide, as it is on the central Natal coast. Off northern Natal and the northeastern Cape the shelf is narrow and inshore current systems are poorly developed. The inshore waters are usually cooler than those of the main current, possibly because of upwelling by cool subsurface water in the centers of the eddies. As a result, animals living on the outer continental shelf and further offshore are usually in warmer waters than will be found inshore at the same latitude; tropical species can thus penetrate further to the south if they are not restricted to inshore waters.

The Natal coast is shown in more detail in Figure 2. The topography of inland Natal should be considered briefly. The central and southern parts are extremely steep, falling from the plateau in Lesotho at an altitude of more than 3000 m to sea level in a horizontal distance of about 250 km. The numerous rivers flow rapidly as a result of this steep topography and the high rainfall of about 80 to 100 cm per year (chiefly during the summer months), and large quantities of sediment are deposited on the continental shelf. The northeastern part of Natal consists of the southern tip of the Mozambique coastal plain and is notable for its low, flat topography and scarcity of rivers. The sea of that part of the coast to the north of St. Lucia (commonly known as the Tongaland coast) is therefore remarkably free of sediments and also has a very narrow continental shelf. The fauna and flora of this region are markedly tropical compared to those of the rest of the Natal coast.

From south of St. Lucia to Durban the continental shelf is wider, with the result that the Agulhas Current is forced to flow further offshore and cooler inshore countercurrents are formed. The amount of sediment shows a spectacular increase, and the relatively cool and dirty water results in a marked drop in the numbers of tropical species as compared to the Tongaland coast.

In southern Natal (to the south of Durban) the continental shelf narrows again and the inshore waters are even cooler, resulting in a marked increase in the numbers of subtropical species.

In the distribution of benthic animals along the east African coast, temperature and oxygen content are probably the most important limiting factors. Although the surface waters of the east coast of southern Africa are
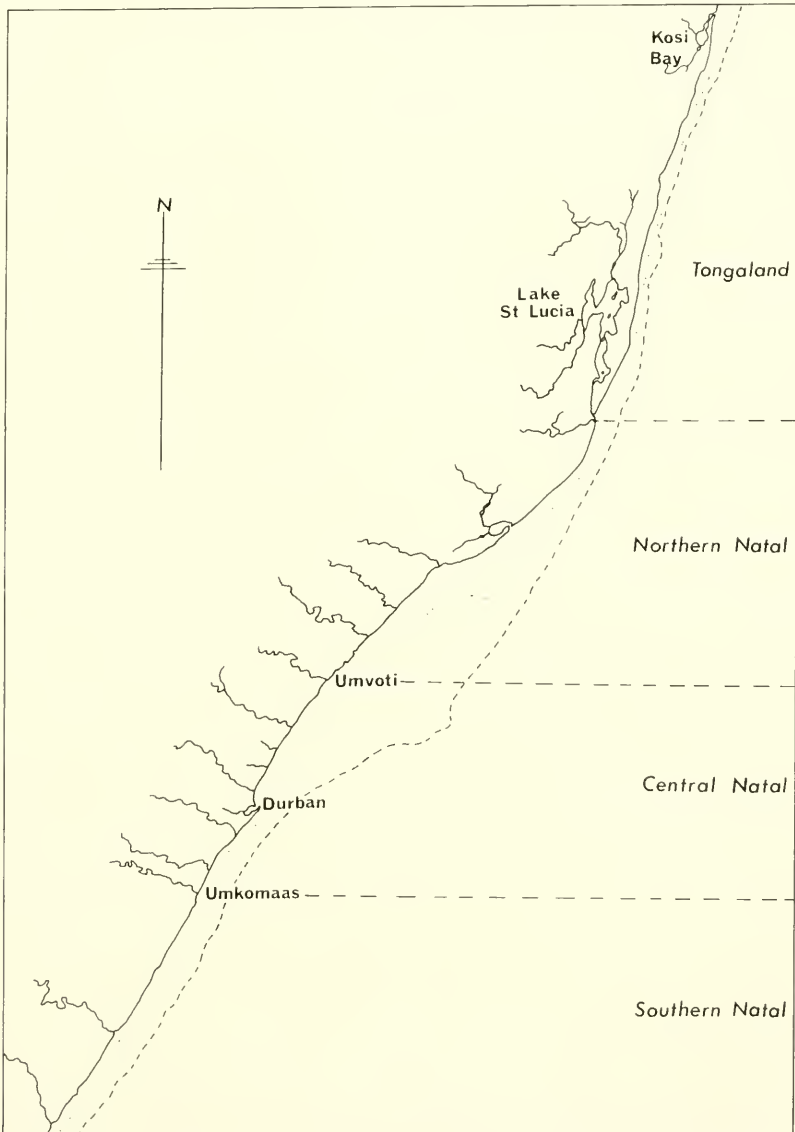
Figure 2 The Natal coast, showing the major rivers entering the sea and the arbitrary subdivisions of the area as outlined in the text. The 20-fathom depth contour is shown as a dotted line, the 100-fathom contour as a dashed line.

generally cooler than those of the northern Indian Ocean, at depths of 200 meters or more the water temperatures are generally higher in the southwestern part than in the north, particularly from 500 m downward. The oxygen content of the subsurface waters also changes significantly along the length of the east African coast, the northern parts being deficient compared to the southern regions (see Wyrtki 1971).

Basic Biogeography of Southern African Seas

Most of our knowledge of the distribution of animals and plants along the southern African coasts is based on Stephenson's (1939, 1944, 1947) work on the intertidal fauna and flora. Further significant contributions have been made by J. L. B. Smith (1949), Day (1967, 1969), and Penrith (1969), among others. An excellent summary of the present state of knowledge is contained in Briggs (1974). The fauna of the southern Cape coast is clearly a warm temperate one derived from the tropical region to the northeast and is distinct in containing a high proportion of tropical species. Briggs suggests the name Agulhas Province for this region and distinguishes it from what he calls the Southwestern Africa Province, which ranges from approximately Cape Point to the tropical boundary in Angola. Water temperatures are cooler in this region than along the southern Cape coasts but not enough to be considered cold temperate. Until more data become available it is probably best to consider this area warm temperate but somewhat cooler and distinct from that to the east of Cape Point.

In this paper the following terms are used, sometimes quite arbitrarily, to refer to different regions:

Southwestern Cape—the western Cape coast to the west and north of the Cape Peninsula

Southern Cape—the southern Cape coast from the Cape Peninsula to Knysna

Eastern Cape—the eastern Cape coast from Knysna to Port St. Johns

Southern Natal—Port St. Johns up to the Umkomaas River

Central Natal—Umkomaas River to Umvoti River

Northern Natal—Umvoti River to St. Lucia Estuary

Tongaland—North of the St. Lucia Estuary to Inhaca Island

Mozambique—Delagoa Bay and northwards

East coast—Knysna to Beira.

DISTRIBUTION OF SHARKS ON THE SOUTHEAST AFRICAN COAST

Until very recently, information on the distribution of sharks along the east coast of southern Africa was at best fragmentary and at worst highly inaccurate. In most cases inadequate knowledge of the taxonomy precluded definition of the ranges of the various species. The present state of knowledge is much improved but still far from complete. In particular, data on sharks occurring on the continental slope and in deeper waters are lacking

while the taxonomy of the squaloid sharks is confused (at least in the present study area) and their distribution can be considered only in general terms. Nevertheless, enough information is at hand to make a first analysis of the distribution of sharks along the east coast of southern Africa.

Adequate description of the distribution of sharks involves more than mere delineation of geographical ranges, as noted by Stewart Springer (1967). Factors such as size and sex distributions in different depths must be considered as well as the interactions of different species in the same area. Since many sharks enjoy nothing more than a meal of other sharks, the adults of small species are subject not only to direct competition from the young of larger species but also to predation by adults of larger species. Further complications may be introduced by species such as *Carcharodon carcharias*, which may have different dentitions, hence different diets, in juveniles and adults.

The most notable feature of the distribution of many shark species is segregation by size and sex. Segregation by sex is apparent even among very young sharks as shown by, for example, *Carcharhinus obscurus* (Bass et al. 1973), *Galeus arae* (Bullis 1967), *Galeorhinus zyopterus* (Ripley 1946), and *Heterodontus portusjacksoni* (McLaughlin and O'Gower 1971). One possible reason for this early segregation by sex is discussed later. Segregation among the larger species is predominantly lateral although some segregation by depth takes place. Among the smaller species segregation is predominantly according to depth, and population differences may occur within relatively short geographic ranges. The larger species usually have wider geographic ranges, with populations at least partly isolated from one another and differing in size, vertebral numbers, litter size and markings plus, probably, many features not easily discerned by human beings.

It is clear that even a basic analysis of the distribution of sharks in a given area is complex. The danger exists that too much attention to detail may result, to put it proverbially, in not being able to see the wood for the trees. The approach here is to treat a few species in detail by way of introduction, then consider the galeoid sharks as a whole. Special attention is paid to the genus *Carcharhinus* as an illustration of the way several closely related species can coexist in one geographical area. The distribution of the nongaleoid sharks is mentioned to complete a picture of the distribution of sharks in the sea off the east coast of southern Africa.

Carcharhinus obscurus

The dusky shark, *Carcharhinus obscurus* (Figure 3), is a relatively large shark known from all three major oceans. Born about 80 to 90 cm long, males mature at about 280 cm and grow to about 325 cm, while females mature at about 300 cm and grow to about 360 cm. Armed with serrated cutting teeth in the upper jaws, *C. obscurus* feeds mostly on bottom-living prey, although it also eats pelagic animals. Elasmobranchs, chiefly demersal forms, are a regular component of its diet.
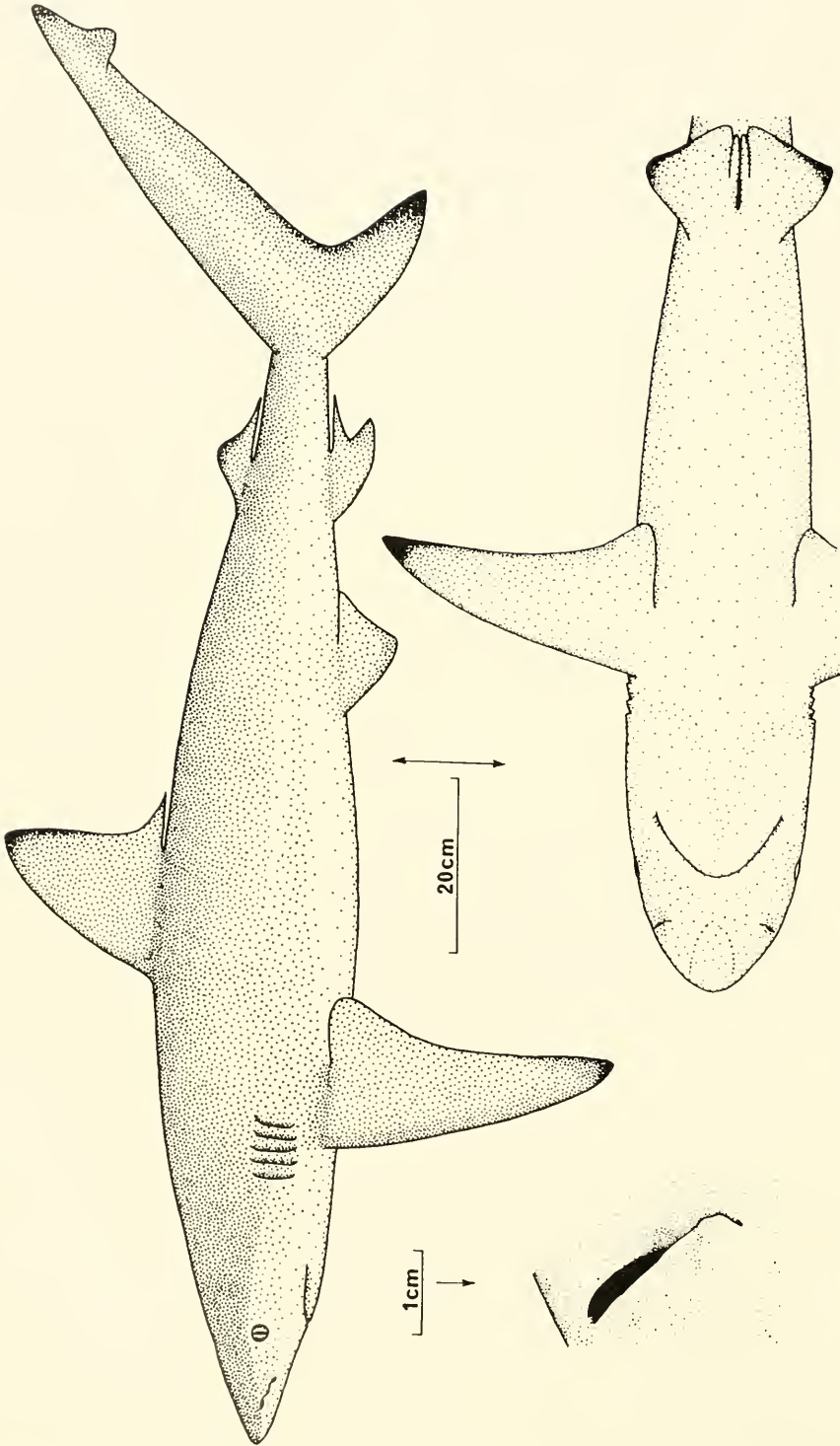
Figure 3   A 165-cm immature male *Carcharhinus obscurus* from Natal. (After Figure 21 of Bass et al. 1973.)

20cm

1cm

In the southwest Indian Ocean *C. obscurus* has a tropical distribution, the home range of the adults being centered in the Mozambique channel where they occur chiefly on the deeper parts of the continental shelves and to a lesser extent close inshore. A fair number of adults, mostly females, range south to Natal and occasionally as far to the southwest as Cape Point. All mating activity takes place in Mozambique waters, as do the early stages of pregnancy. Most of the females found in Natal waters are in the late stages of pregnancy or have recently given birth. There is no marked peak in the numbers born at any one time, but there may be an increase in births from April until June.

As the young sharks grow they tend to move out of this area, most of the young males moving to the southern Cape coast and most of the females to the northern Natal coast and, to a lesser extent, the Tongaland and southern Mozambique coasts. As they grow, young dusky sharks seem to move into deeper water and then northward. *C. obscurus* up to 220 cm long are concentrated off the southern Natal coast but definitely further offshore than the areas where very young (80 to 130 cm) sharks are caught. Above this size range, most of the sharks seem to move northward into Mozambique waters.

The movements of the young (less than 120 cm) dusky sharks are worth considering in greater detail. Much of the information on these movements came from a tagging program based in Durban (see Davies and Joubert 1966; Bass et al. 1973). Quite significant sexual segregation is apparent in these immature sharks, varying according to locality as shown in Table 2.

The numbers of sharks examined from southern Natal and the Cape coasts were rather small, but further evidence of segregation is seen in Table 3, which documents the recaptures of *C. obscurus* tagged and released at Durban.

The biased dispersal of the tagged sharks (females tending to move northward and males southward) is marked. This dispersal is primarily a migration rather than a gradual movement, particularly in the case of the sharks recovered in the southern and eastern Cape. Ten of those recovered in this region had moved at speeds of at least 16 km (10 mi) per day (calculated from the day of release to that of recapture). Proof that the sharks may stay in the Durban area for some time before migrating is shown by one tagged and released in Durban during September, recaught and rereleased in the

Table 2. Sexual segregation of immature *C. obscurus.**

Locality	Males	Females	Percentage of Males
Central and northern Natal	940	1599	37.0
Southern Natal	25	21	54.4
Eastern and southern Cape	31	21	59.6

*After Table 46 of Bass et al. (1973).

Table 3. Recaptures of *C. obscurus* tagged and released at Durban.*

Recapture Locality	Number	Males	Females	Percentage of Males
More than 50 km north of Durban	10	1	9	10
50 km or less north of Durban	26	7	19	27
Tagged at Durban	2174	804	1370	37
Natal, south of Durban	23	9	14	39
East and south Cape coasts	39	19	20	49

*After Table 47 of Bass et al. (1973).

same area during December of the same year, and then recaptured in the eastern Cape one month later. There is also evidence (see Bass et al. 1973) that the number of recaptures from the eastern Cape is fewer than would be expected of a gradual movement down the coast, and the conclusion is that these sharks do not usually feed inshore while migrating and that the migrations tend to be quick, nonstop movements rather than a gradual drifting along the coast.

The sharks migrating long distances tend to be larger than those staying in the Durban area for some time—a few sharks have been recovered in the Durban area 1 and even 2 years after tagging. Apart from this sex-related dispersal there is a north-south movement that can be correlated with seasonal temperature changes, the population as a whole moving to the north as the water cools in winter and then moving southward again in summer. During the warmest months an offshore movement to deeper and presumably cooler waters seems to take place. The seasonal distribution of young dusky sharks from inshore waters has a marked correlation with temperature, as shown in Figure 4; the preferred temperature range is 19° to 23°C.

One certain effect of the size segregation in *C. obscurus* is that the young sharks are kept away from the adults until they are large enough not to be tempting prey for their elders. Sexual segregation, as occurring in the adults, is easily explained: there is no need for the adult males to come anywhere near the nursery areas. With immature sharks the phenomenon of sexual segregation is not so easy to explain. Bass et al. (1973) suggest that

> ... the young *C. obscurus* are genetically dimorphic with regard to the direction they migrate in as they grow. This would result in a spreading out of these sharks over a wide section of the coast (the secondary nursery area) despite a comparatively small primary nursery area (the southern Natal coast), although why the latter area should be so small is not apparent at present. In this way population pressure would be reduced and unsuitable conditions in any part of the range would only affect part of the population. The sexual segregation could have evolved in response to the northern Natal coast being, on the average, the most suitable area for the survival of young *C. obscurus.* Little is known about
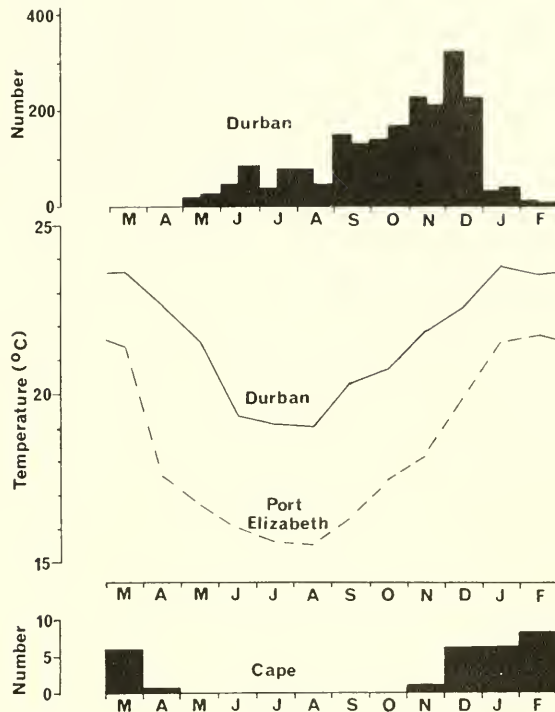
Figure 4   Seasonal distribution of *Carcharhinus obscurus* juveniles tagged and released in Durban and of those recovered from the Cape coast to the southwest of East London, together with the mean surf temperatures in Durban and Port Elizabeth. (After Figure 23 of Bass et al. 1973.)

the mating habits of sharks but it is unlikely that they form extended pair-bonds and a relatively small proportion of adult males may be able to serve a high proportion of females. This is particularly likely in galeoid sharks where in many cases the females mate at most every two years, a situation which holds true for *C. obscurus*. It would thus be advantageous for the bulk of the female young to grow up in that part of the range where they stand the best chance of survival. The movement of males into less suitable parts of the range may be a result of selection for an even spread of the population over the secondary nursery area despite a tendency for the females to move into a limited part of the region. The genetic dimorphism resulting in a spreading out of the sharks from the primary nursery area, which may have evolved to avoid competition between the newborn and the slightly older animals, would thus be partially linked with sex.

Carcharhinus leucas

The Zambezi or bull shark, *Carcharhinus leucas* (Figure 5), is a well-known species found in all three major oceans and recorded from many freshwater localities. Specimens have been recorded along the east coast of Africa from Mombasa to central Natal. *C. leucas* is slightly smaller than *C. obscurus*, and maturity takes place at about 225 cm in both sexes, which reach a maximum length of about 300 cm. The young are born about 60 to 70 cm long. These figures refer to the southeast African population. In other parts of the world *C. leucas* may be considerably smaller. For instance, males from Lake Nicaragua mature at 160 to 170 cm (Thorson et al. 1966). The teeth of *C. leucas* are similar to those of *C. obscurus*, being well adapted for cutting and enabling this shark to feed on relatively large prey. In addition to feeding on live prey, *C. leucas* is something of a scavenger and has been held responsible for several attacks on human beings. Smaller sharks and other elasmobranchs form a good part of its diet.

C. leucas is best known for its ability to enter freshwater, which has given rise to the common name of Zambezi shark in southern Africa where it has been recorded more than 1000 km from the sea in the Zambezi river system. The distribution is basically tropical, with adult sharks found mainly in the shallow waters of northern Natal and the warmer regions to the north. The population off southern and central Natal consists mainly of adolescent sharks ranging from 150 to 230 cm plus a few adults and a small proportion of juveniles of less than 150 cm. There are no records of this species south of Natal, although it is quite likely that occasional specimens range down to the eastern Cape during the summer months. No adult females with ripe ova or with young embryos have been recorded from Natal, and it seems that (as in the case of *C. obscurus*) all mating activities and the early stages of pregnancy take place in tropical waters.

The size distribution in freshwater systems is quite different from that in the sea. *C. leucas* has been recorded from most of the river and lake systems of the east African coast from the Zambezi to Durban Bay. The bulk of these records are of juvenile and adolescent sharks, as shown in Figure 6, which compares the size distributions of *C. leucas* taken in the sea off southern and central Natal with those taken in different parts of the Lake St. Lucia system in northern Natal. This system consists of an H-shaped lake some 40 km long and about 20 km across at its widest point, connected with the sea by a channel some 20 km in length (commonly known as the Narrows).

A tagging program on the sharks in this system showed them to move freely between the sea and the main lake—one animal was tagged in the main lake, recaught in the Narrows near the sea after four days, then caught again in the main lake three weeks later. The only adult sharks caught in the St. Lucia system were four large females taken close to the mouth of the estuary. Only two were examined internally; one proved to be pregnant with full-term embryos while the other had recently given birth.
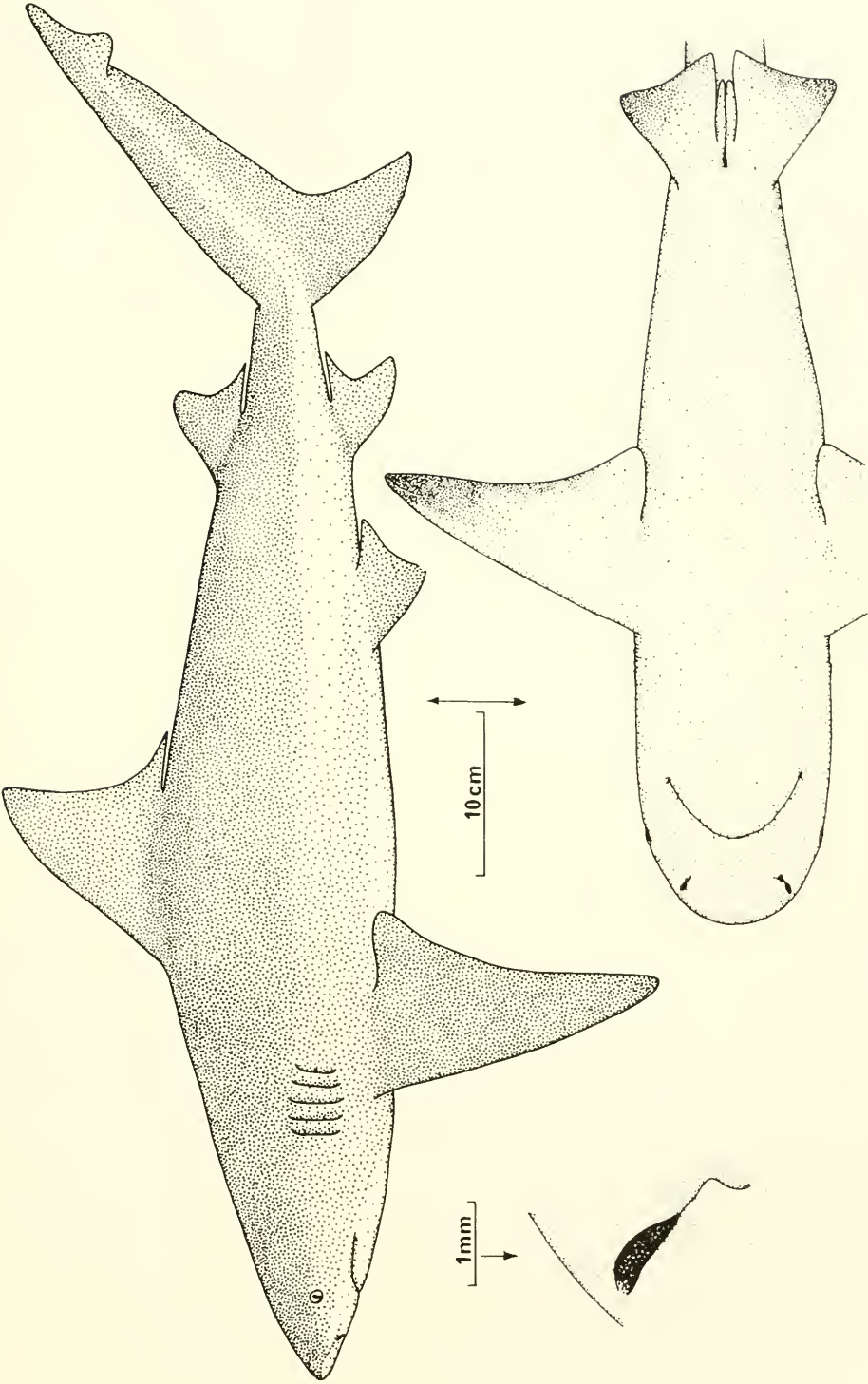
Figure 5 A 166-cm immature male *Carcharhinus leucas* from Natal. (After Figure 13 of Bass et al. 1973.)
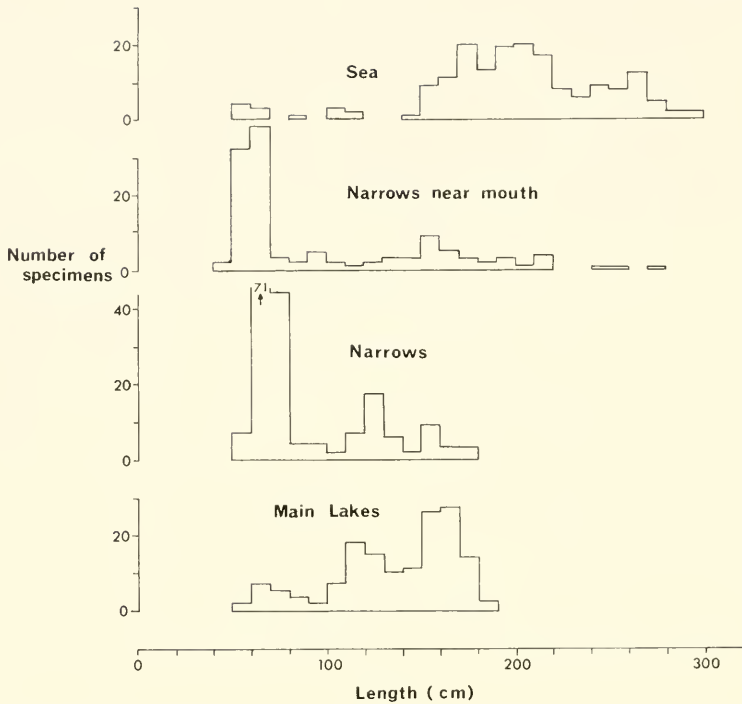
Figure 6 Size distribution of *Carcharhinus leucas* taken in different
parts of the St. Lucia system and in the sea off central and southern
Natal. (After Figure 15 of Bass et al. 1973.)

> It seems that adults usually avoid entering the system but pregnant
> females may make short forays into the estuary, probably to give birth.
> Many of the juveniles caught in the estuary and Narrows had open or
> newly healed umbilical slits, showing that they had been born not long
> before. (Bass et al. 1973)

Somewhat larger sharks, between 110 and 200 cm in length, were prepon-
derant in the main lake. In addition, there was a predominance of females
in the main lake and of males in the Narrows.

It appears that young *C. leucas* are born in the estuary where they tend to
stay for the early part of their lives. As they grow they tend to move into the
main lake and then out to sea as they mature. At all ages some movement
between the sea and the lake system probably takes place and, in particular,
sharks of 90 to 120 cm are rare in the Narrows. Further work might show
seasonal changes in the distribution of *C. leucas* in the St. Lucia system.

As noted by Bass et al. (1973),

> The size distribution of *C. leucas* in systems such as the Zambezi river
> and its tributaries may differ from that observed in St. Lucia. Adult
> sharks are found in Lake Nicaragua where Thorson et al. (1966) recorded
> adult males. However, Thorson (1971) has recently proved that these

sharks travel between the sea and Lake Nicaragua and this population is not landlocked. There are other parallels between the sharks in Lake Nicaragua and in St. Lucia. Thorson (personal communication) notes that the population of *C. leucas* near the mouth of the Rio San Juan (which connects the lake to the sea) includes a large number of juveniles about 50 to 80 cm in length. The 90- to 100-cm size class is scarce but above that size they become common again.

A further peculiarity of the St. Lucia system is that it may become hypersaline during times of drought when evaporation exceeds the inflow of freshwater so that the level is maintained by seawater entering by the Narrows. Salinities approaching 100‰ have been recorded in the past. The sharks appeared to be partly at home in moderately hypersaline waters, evidence showing that only salinities greater than 50‰ were avoided. During this time most of the sharks in the lake were in noticeably poor condition, despite the facts that food (teleost fish) was abundant and the sharks were not confined to the lake but regularly traveled between it and the sea. Possibly the extra stress of coping with the high salinities was the cause of the poor condition.

Odontaspis taurus

Commonly known as the ragged-tooth or sand shark, *Odontaspis taurus* (Figure 7) is another large, wide-ranging shark with a tropical distribution. Born at a length of about 100 cm, males mature at about 210 cm and females at 230 to 240 cm. Maximum length is about 260 cm in males and about 290 cm in females from the southwest Indian Ocean. Armed with a conspicuous set of lanceolate teeth in upper and lower jaws, *O. taurus* feeds mainly on fish and small sharks, most of which are swallowed whole. The several attacks on humans for which it has been blamed were usually based on flimsy evidence (see Baldridge 1973 and Davies 1960, 1964). As Bass et al (1975c) point out, ". . . the habits and dentition of *O. taurus* makes it an unlikely maneater except possibly at times when factors such as shot fish may cause unusual excitement."

In the southwest Indian Ocean *O. taurus* has been recorded from shallow inshore waters ranging from the southern Cape to southern Mozambique. Rather sluggish, it is usually found near reefs. It exhibits a remarkable method of buoyancy control by means of air swallowed at the surface and held in the stomach (Bass and Ballard 1972). Unlike the faster swimming species of the genus *Carcharhinus*, *O. taurus* is able to pump sufficient water over its gills to satisfy its respiratory requirements while at rest. This method is also used while swimming slowly, but when above a certain speed this shark relies solely on its movement through the water to ventilate the gills.

O. taurus has distinct segregation and migration patterns. As with many other tropical species, mating and the early part of pregnancy takes place in warm waters, in this region in southern Mozambique and off the Tongaland coast. Each winter the pregnant females migrate southwards along the coast, the peak of this movement passing Durban in July and the southern Natal coast during August and September. The young are born in the eastern Cape, after which a slower movement back to the north takes place, passing the
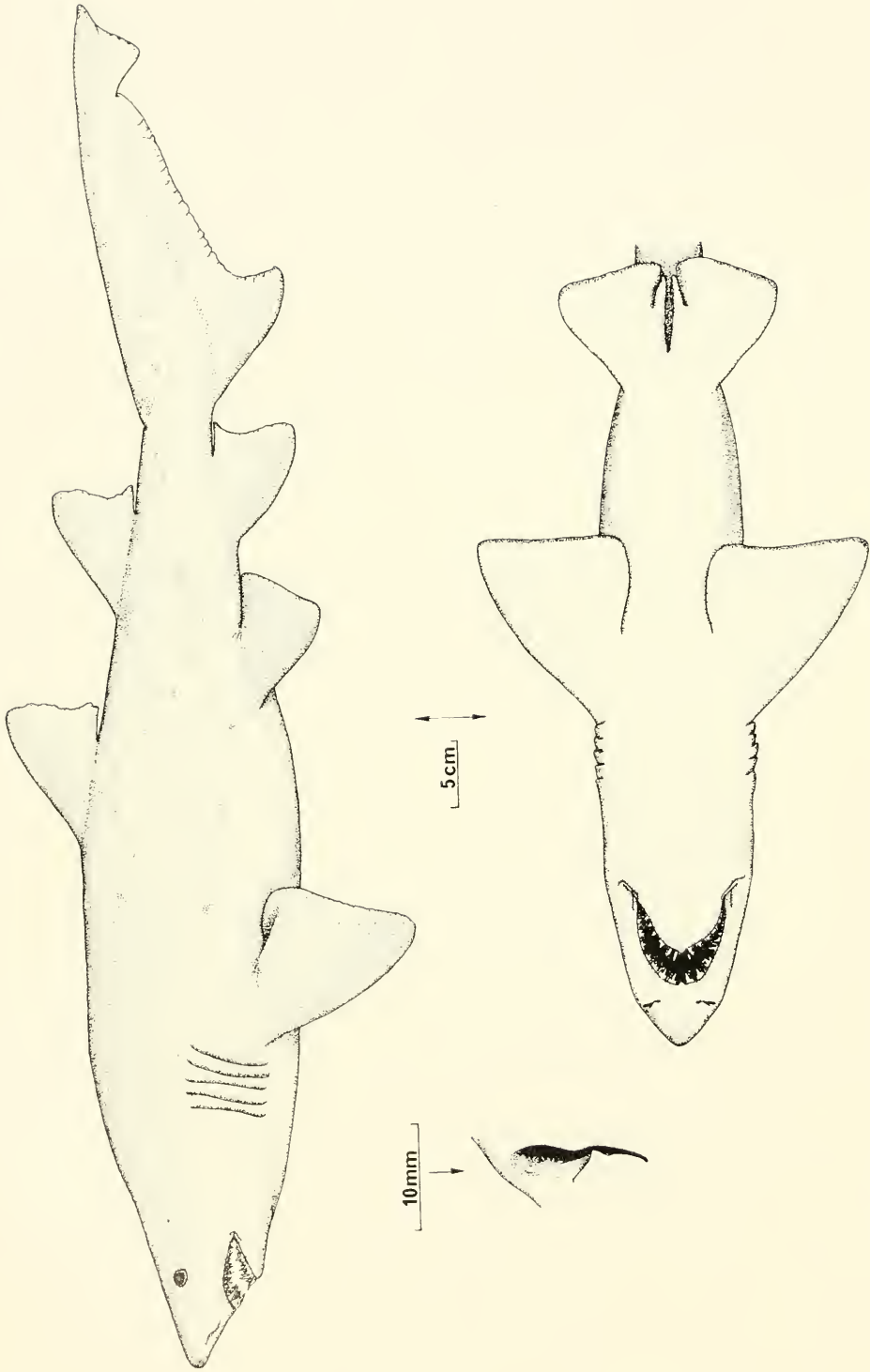
Figure 7   A 103-cm immature male *Odontaspis taurus* from the eastern Cape. (After Figure 7 of Bass et al. 1975c.)

central Natal coast from September to November. The young sharks stay in the eastern and southern Cape, moving further to the north only when they attain a length of about 180 to 200 cm. The segregation of juvenile and adult *O. taurus* is quite noticeable in the size distributions of specimens taken from the Natal and eastern Cape coasts, shown in Table 4.

Table 4. Size distribution of *O. taurus* taken from the Natal and eastern Cape coasts.*

Capture Locality		Total length (cm)						
		< 130	130–159	160–189	190–219	220–249	250–279	> 280
Natal	Male	0	0	0	2	8	1	0
	Female	0	0	2	0	13	42	1
Eastern Cape	Male	6	5	0	1	0	0	0
	Female	3	1	4	2	1	1	0

*After Table 4 of Bass et al. (1975c).

Carcharodon carcharias

The great white shark, *Carcharodon carcharias* (Figure 8), is one of the most widely recognized species because of its relatively large size and proven habit of occasionally including *Homo sapiens* in its diet. The size attained by members of the different populations of *C. carcharias* probably varies—males from the southwest Indian Ocean are mature at 300 cm in length but those from Florida are immature at about 380 cm (Bigelow and Schroeder 1948). The usual maximum length seems to be about 4 to 5 m for males and about 6 m for females. Randall (1973) notes that the largest reliable record of this species is a 6.4-m shark from Cuba.

Bass et al. (1975c) note that

> *C. carcharias* is commonly reputed to be a tropical oceanic species but in actual fact is normally found on the continental shelf, often close inshore. While the adult sharks may occasionally visit the tropics, juveniles appear to inhabit warm temperate or temperate waters which are also the regions most commonly frequented by the larger specimens. In the seas about southern Africa the home range of *C. carcharias* appears to be the southern and southwestern Cape coasts. In the warmer waters of Natal the species is a regular visitor, and a large specimen has been recorded from the Seychelles. On a worldwide basis the species has been recorded from the warm temperate, subtropical, and tropical parts of all the major oceans, including the Mediterranean.

The distribution of this shark in Natal waters is summarized in Figure 9, from data provided by Wallett (1973) on catches in protective nets set near bathing beaches. These data are divided in two arbitrary groups, large (more than 2.4 m) and small (less than 2.4 m), and into two geographical areas, the south and north of Durban. In the northern area the younger sharks show a
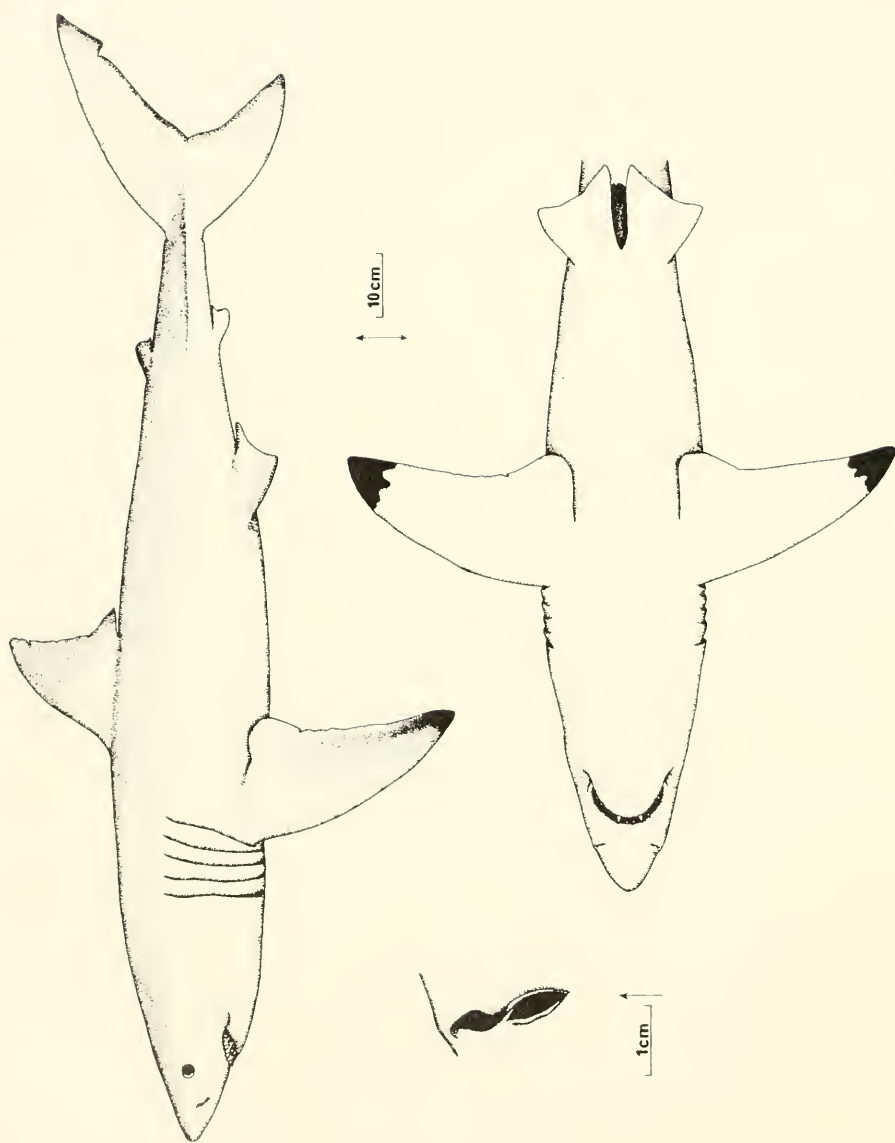
Figure 8   A 177-cm immature female *Carcharodon carcharias* from Natal. (After Figure 10 of Bass et al. 1975c.)
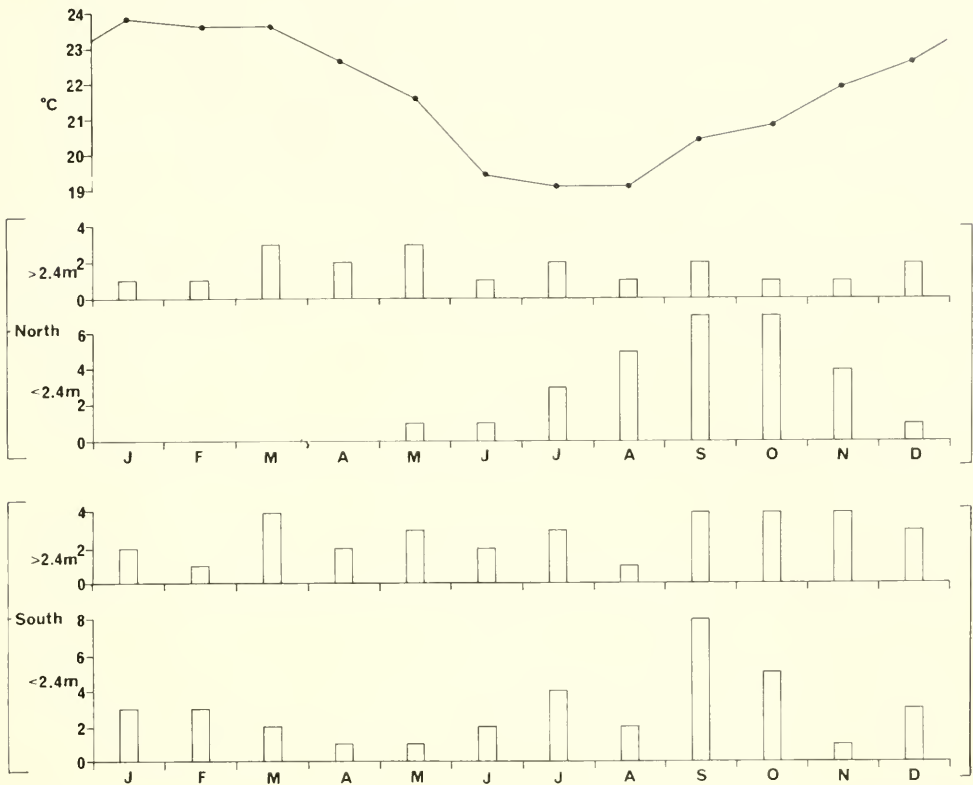
Figure 9 Seasonal distribution of 117 *Carcharodon carcharias* taken in the shark nets off Natal, divided into two arbitrary regions north and south of Durban. (After the data given in Figure 38 of Wallett 1973.) Also shown are the monthly surf temperatures as recorded at Durban. (After Figure 12 of Bass et al. 1975c.)

pronounced seasonal occurrence with a peak in September and October. Larger sharks occur in lesser numbers throughout the year with a slight peak from March to May. In the southern part of Natal *C. carcharias* is definitely more abundant, the younger sharks being found throughout the year with only a slight peak in September and October. The larger specimens again occur throughout the year without any marked seasonality.

> Bearing in mind that water temperatures are lower in southern Natal as compared to the northern area, it seems clear that young *C. carcharias* have a warm temperate rather than a tropical or subtropical distribution. In Durban and northwards these juveniles are caught only during the cooler parts of the year. To the south of Durban, however, water temperatures are somewhat lower and these young sharks are caught throughout the year. Although we have few definite records, small *C. carcharias* appear to be fairly common in southern and southwestern Cape waters. (Bass et al. 1975c)

The size and seasonal distributions of *C. carcharias* caught in the Durban area (summarized in Figure 10) make the segregation of small and large specimens clear. Large sharks occur principally in the first half of the year and smaller sharks in the second half of the year with a peak at about August or September. This distribution reflects some movement or migration other than that caused by seasonal temperature changes, otherwise more large sharks should be caught during the period October to December, when water temperatures are roughly equivalent to those of March to May. *C. carcharias* differs from most of the large sharks found in the seas about southern Africa in having a range that is basically warm temperate rather than tropical. The actual nursery areas have still to be defined. As yet, virtually nothing is known about the breeding habits.

Scylliogaleus quecketti

Scylliogaleus quecketti (Figure 11) is a small crustacean-eating shark with demersal habits. Born at a length of about 34 cm, males are mature at 70 cm and females at 80 cm with a maximum size of slightly more than a meter. All known specimens of this species have come from shallow water off the Natal and eastern Cape coasts, the great majority being adolescent or adult females. Many of the latter have contained embryos, some full term, others still in the earliest stages of development. Only two males, both adult, have been recorded and the habitat of juvenile sharks of both sexes is unknown. Presumably it is in deep water away from the shore, possibly among reefs where sampling by means of trawls is not possible. It is unlikely that a
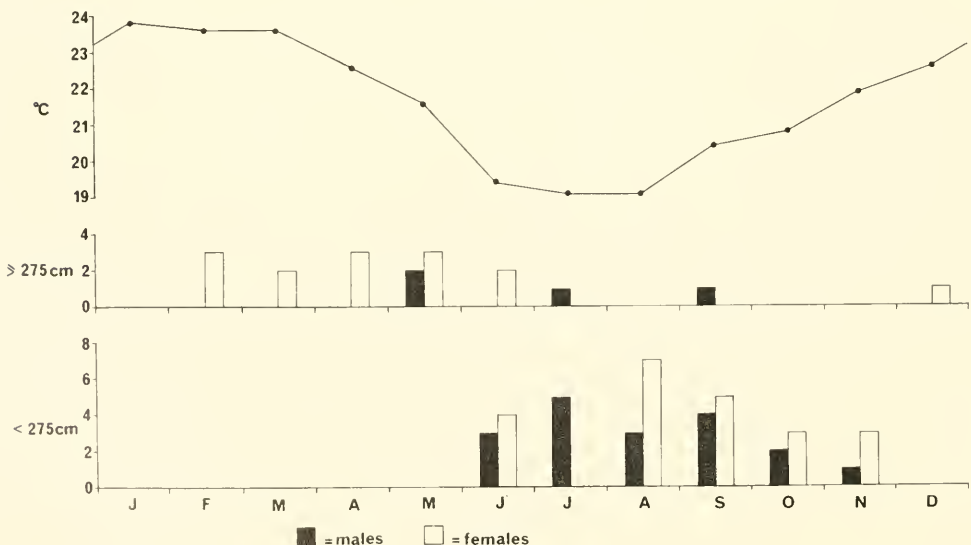
Figure 10   Seasonal distribution of 58 *Carcharodon carcharias* taken in Natal waters (mostly from the Durban area). Also shown are the monthly surf temperatures as recorded at Durban. (After Figure 11 of Bass et al. 1975c.)

10 cm
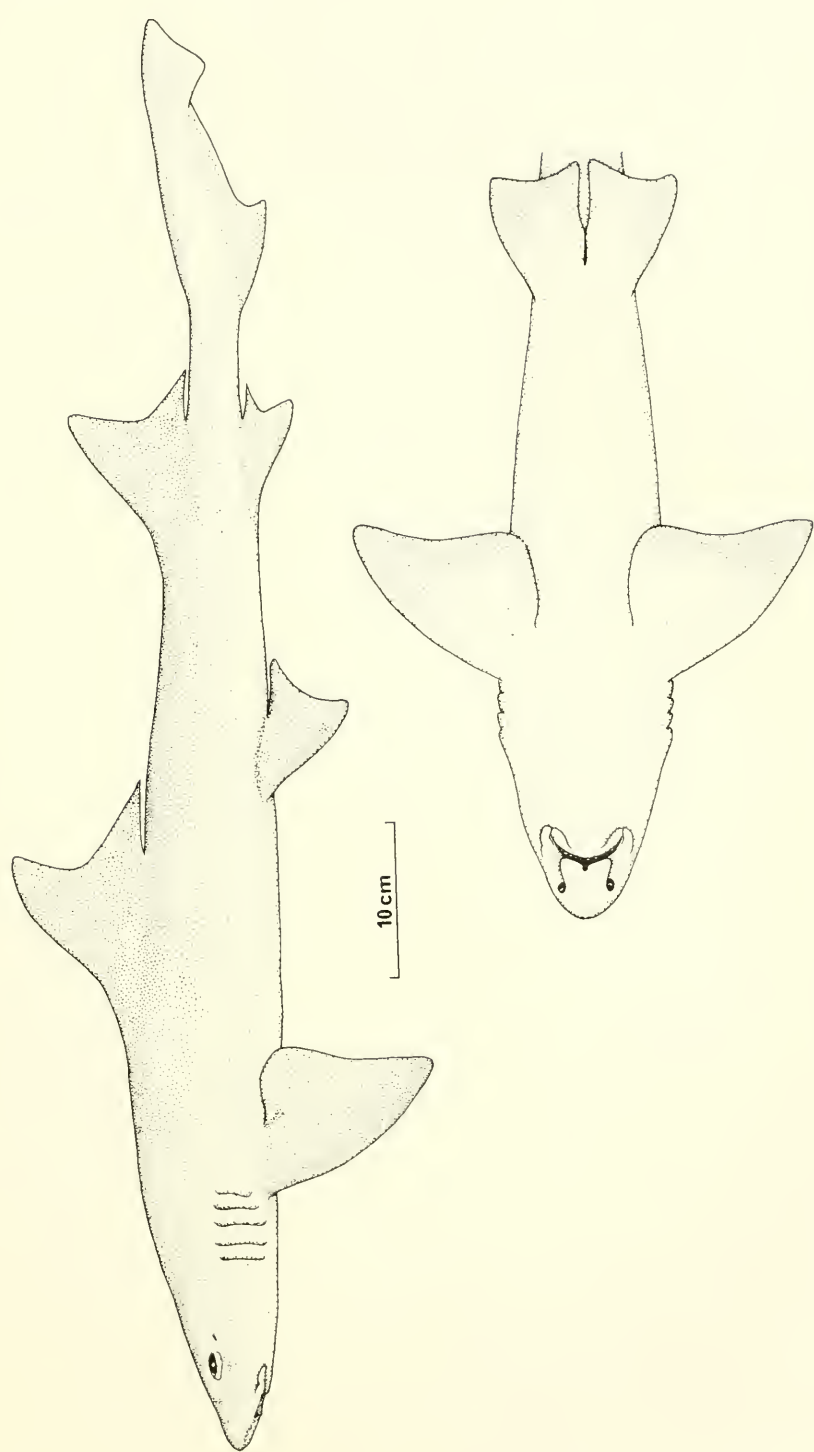
Figure 11   A 94-cm adult female *Scylliogaleus quecketti* from Natal. (After Figure 8 of Bass et al. 1975b.)

slow-swimming shark of this size makes extensive migrations along the length of the southern African coastline when a relatively short movement away from the coast results in a marked change in environment.

Holohalaelurus regani

Holohalaelurus regani is a small catshark found on the outer parts of the continental shelf and on the continental slope along the south and east coast of Africa from the southwestern Cape to East Africa. As shown in Figure 12, specimens from the southwestern Cape can be distinguished from those found off Natal. Those found off Natal are smaller than those from the Cape, with males maturing at 45 to 50 cm and attaining a length of at least 55 cm and females maturing at 38 to 39 cm and growing to about 45 cm. In contrast to most sharks, the males are larger than the females, a feature also of the other member of the genus, *H. punctatus*.

Another striking feature of this bottom-living, oviparous species is that the young apparently live on the continental slope in deeper water than that normally occupied by the adults. Young *H. regani* also are markedly different from their elders in appearance (Figure 13), being dark black in color both dorsally and ventrally. "The overall dark colouration is reminiscent of that found in deep-water squaloid sharks such as the genus *Etmopterus* and may indicate similar habits." (Bass et al. 1975a).

The Genus Carcharhinus

The genus *Carcharhinus* includes 28 species (Garrick 1967), of which 17 have been recorded from the southwestern Indian Ocean (Bass et al. 1973). All are fairly fast-swimming pelagic species, ranging in length from about 1 to 4 m and armed with flattened cutting teeth. Of the 17 species from the region, *C. galapagensis* has been found only at the Walters Shoal while *C. amblyrhynchos* has been recorded only once (from the northwest coast of Madagascar). The remaining 15 species are listed in Table 5 in descending order of size as estimated from the lengths at maturity given by Bass et al. (1973, Table 5C).

Of these 15 species only *C. longimanus* is not a continental shelf inhabitant, although even relatively small inshore species such as *C. sealei* and *C. melanopterus* have been caught over deep water on occasion. *C. longimanus* has a tropical offshore distribution and is commonly found over deep water off Durban; it also ranges far southward in warm Agulhas Current water. For instance, two specimens were taken some 400 km south of Cape Agulhas where the surface water temperature was 21.3°C.

Thus, 14 species are normally found on the continental shelf. Most have tropical distributions, only *C. brachyurus* having a warm temperate range centered in the southern and southwestern Cape. This shark is caught in the eastern Cape throughout the year and ranges north to southern Natal (occasionally to central Natal) in the winter months. *C. altimus* is markedly different from all the other species in that it is usually caught on or near the
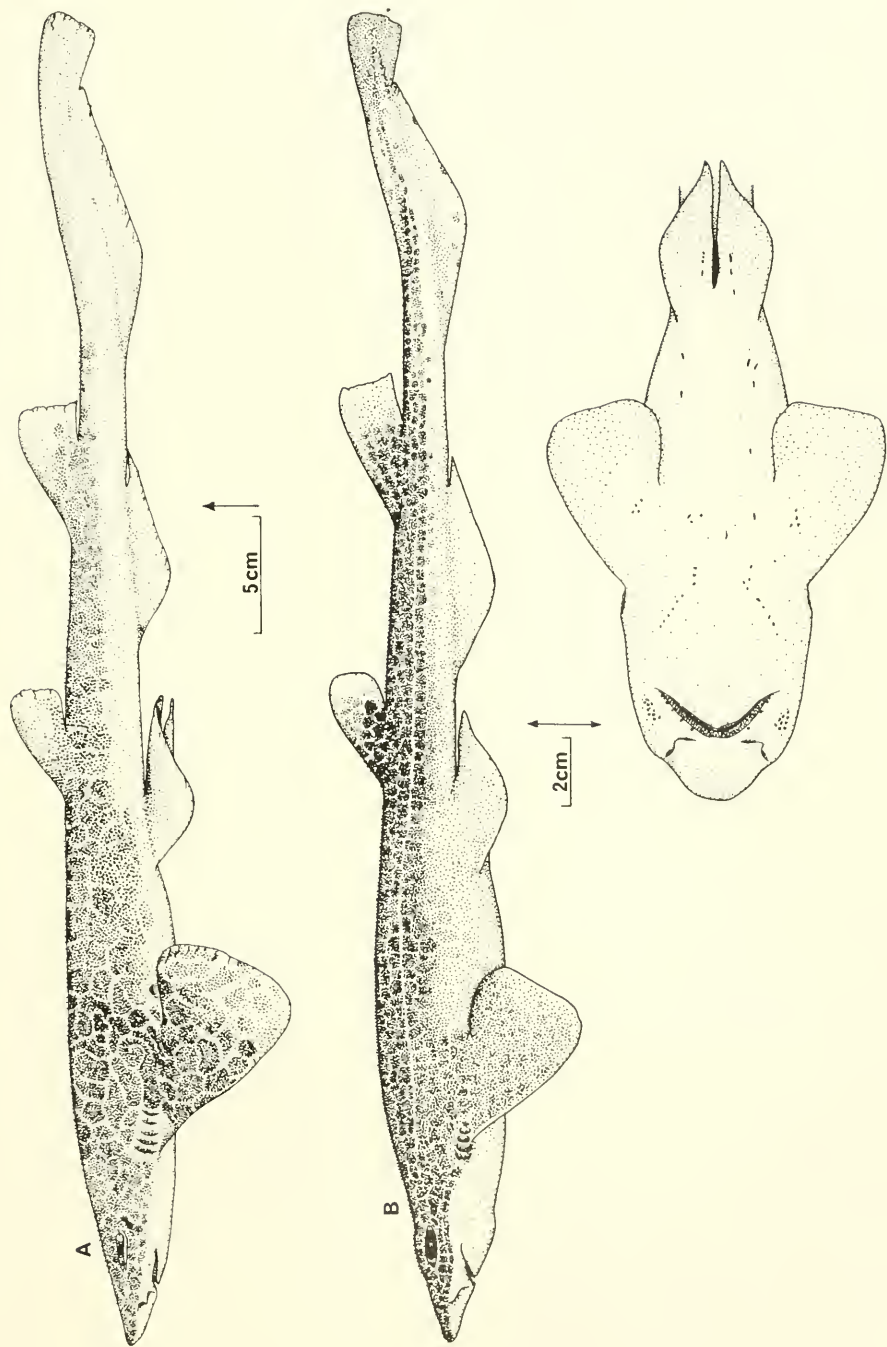
Figure 12 *Holohalaelurus regani*: (A) A 57-cm mature male from the southwestern Cape and (B) a 39-cm mature female from Natal. (After Figure 15 of Bass et al. 1975a.)
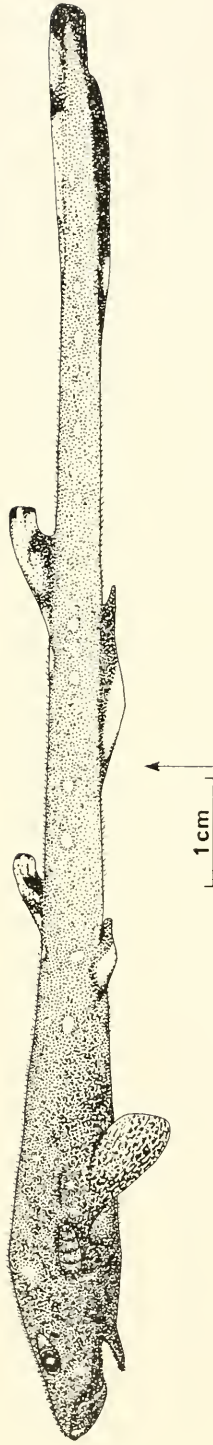
Figure 13   A 13-cm immature female *Holohalaelurus regani* from Natal. (After Figure 15 of Bass et al. 1975*a*.)

Table 5. Fifteen species of *Carcharhinus* recorded from the southwest
Indian Ocean.*

| | | Total lengths (cm) | |
Species	Birth size	Size at maturity	Maximum size
C. obscurus	80–90	270–290	360
C. leucas	60–70	220–240	300
C. falciformis	75–85	200–240?	300?
C. brachyurus	60–70	200–220	290
C. amboinensis	70–75	190–210	280?
C. altimus	75–90	210?	280?
C. longimanus	60–65?	190–200	270
C. brevipinna	65–75	170–200	270
C. albimarginatus	70–80	170–190	250
C. limbatus	60–65	170–180	250
C. plumbeus	60–65	160–170	210
C. spallanzani	65–75	110–120	170
C. sorrah	50–60	110–120	160
C. melanopterus	45–55?	90–110	180
C. sealei	35–45	70–80	100

*In descending order of size as estimated from lengths at maturity given by Bass et al.
(1973) in Table 5C.

seabed on the deeper parts of the continental shelf. Two other species, *C. obscurus* and *C. falciformis*, are also normally found over the outer continental shelf but usually in open water rather than near the bottom. Both of these are large sharks, with *C. falciformis* having a more strictly tropical distribution. This shark rarely ranges into Natal waters and presumably has its nursery grounds in deep water off the southern Mozambique coast. Nursery grounds of this species have been identified only in the northwest Atlantic, where the newborn young are found near the bottom along the outer edge of the continental shelf (Springer 1967). *C. obscurus*, on the other hand, uses shallow water off the Natal and southeastern Cape coasts as nursery areas. Adult *C. obscurus*, chiefly pregnant females, are common off Natal and range as far southwest as Cape Point.

The 10 remaining species can be classified as inshore-dwelling sharks. These are *C. albimarginatus, C. amboinensis, C. brevipinna, C. leucas, C. limbatus, C. melanopterus, C. plumbeus, C. sealei, C. sorrah,* and *C. spallanzani.* The ecological separation of these sharks is difficult to define in some cases and appears to involve depth, feeding, and the use of different nursery areas. They can be divided into three groups according to size:

Those maturing at less than 150 cm	*C. sealei* *C. melanopterus* *C. sorrah* *C. spallanzani*
Those maturing between 150 and 200 cm	*C. brevipinna* *C. limbatus* *C. plumbeus* *C. albimarginatus*
Those maturing at more than 200 cm	*C. leucas* *C. amboinensis.*

C. amboinensis and *C. leucas* are relatively large, slow-swimming species armed with broad erect cusps on the teeth of their upper jaws. *C. plumbeus* has similar teeth but is somewhat smaller and possibly slightly faster. *C. albimarginatus*, *C. brevipinna*, and *C. limbatus* are sleek, speedy species with erect, relatively narrow-cusped teeth while *C. sealei*, *C. melanopterus*, *C. sorrah*, and *C. spallanzani* are small to medium-sized sharks with obliquely cusped teeth.

C. amboinensis and *C. leucas* are remarkably similar in external appearance but are easily distinguished by their different habitat preferences, at least on the African coast. *C. leucas* is commonly found in very shallow coastal waters while the young spend at least part of their lives in rivers and inland lake systems. *C. amboinensis* is found in slightly deeper waters off the African coast. The nursery areas for this population are not known; almost all the specimens taken off Natal have been immature but well above birth size. At Madagascar a different situation occurs. There *C. leucas* appears to be absent and *C. amboinensis* is found in very shallow water and possibly also in freshwater. The two sharks appear to be complementary to each other in that *C. leucas* is dominant off the east African coast while *C. amboinensis* is dominant near Madagascar and possibly also near the offshore islands of the southwest Indian Ocean.

The four medium-sized inshore species can be split into two groups according to their dentition. *C. brevipinna* and *C. limbatus* have relatively narrow cusps to their teeth and are apparently adapted to feeding on smaller prey than *C. plumbeus* and *C. albimarginatus*, which have broader cusps on the teeth of their upper jaws. Differences in the ecological preferences of *C. plumbeus* and *C. albimarginatus* are not clearly defined in present data. *C. albimarginatus* appears to be faster swimming and may inhabit, on the average, shallower water than *C. plumbeus*. In addition, *C. albimarginatus* is confined to clear water (it does not occur south of Tongaland), while *C. plumbeus* juveniles (but not newborn young) are fairly common in the relatively dirty water of the northern and southern Natal coasts. For some reason this shark is virtually absent from the central Natal coast but fairly common on the northern and southern Natal coasts, where it is usually caught over muddy bottoms. The only clear difference between *C. limbatus* and *C. brevipinna* is their use of separate nursery areas. The Natal coast is commonly

used as a primary nursery gound by *C. brevipinna* but only rarely by *C. limbatus*, which probably drops its young off Tongaland or southern Mozambique. To complicate the issue, somewhat older (but still immature) *C. limbatus* are not uncommon in Natal where *C. brevipinna* of a similar age are rare. It seems possible that *C. brevipinna* of this age group live to the south of Natal.

The four remaining sharks (*C. sealei*, *C. melanopterus*, *C. sorrah*, and *C. spallanzani*) are relatively small species with narrow, oblique cusps to the teeth of both jaws. *C. sealei* is by far the smallest *Carcharhinus* species of the region, maturing at about 75 cm and rarely attaining more than a meter in length. It appears to tolerate a wider range of environmental conditions than the other three small species, none of which ranges south of Tongaland. *C. sealei* has been recorded throughout the tropical parts of the southwest Indian Ocean and is not uncommon in the seas of northern and central Natal. In coral reef areas it seems to be less abundant than the other three sharks. In such conditions *C. melanopterus* is abundant in the very shallow waters of the lagoons and the channels joining the lagoons to the open sea. The slightly larger *C. sorrah* prefers the shallowest waters outside the lagoons, while the even larger *C. spallanzani* inhabits somewhat deeper waters, only the juveniles regularly coming into very shallow areas. The latter species is common on the Tongaland coast while the other two are more strictly tropical and do not occur to the south of Delagoa Bay.

The Carcharhiniform Sharks

The genus *Carcharhinus* cannot be considered completely on its own, for many other sharks live on the east coast of southern Africa, some of them competing in one way or another with the various species of *Carcharhinus*. For a start we can consider the group commonly known as the carcharhiniform sharks. This includes the sharks of the families Scyliorhinidae, Pseudotriakidae, Carcharhinidae, and Sphyrnidae. These four families are not clearly demarcated from one another but instead form arbitrary divisions in a morphological gradient ranging from sluggish, bottom-living types (scyliorhinids) through faster but demersal types (*Pseudotriakis* and the "lower" carcharhinids) to fast pelagic forms inhabiting open water ("higher" carcharhinids and the sphyrnids).

Sphyrnids and "Higher" Carcharhinids—Apart from the genus *Carcharhinus*, nine "higher" carcharhinids and three sphyrnids are found on the east coast of southern Africa (see Bass et al. 1975*b*). The general pattern of distribution is much the same as in *Carcharhinus*, with a predominance of tropical species living in inshore waters. Among the carcharhinids there is one offshore species (*Prionace glauca*) with a temperate distribution as compared to the tropical *Carcharhinus longimanus*. A further inshore species (*Galeorhinus galeus*) has a temperate distribution ranging from the southwestern to the eastern Cape. This is a medium-sized shark maturing at 120

to 140 cm. The seven remaining species, all having tropical distributions, divide by size as follows:

Maturing at less than 150 cm	*Rhizoprionodon acutus* *Loxodon marcrorhinus* *Hypogaleus hyugaensis* *Triaenodon obesus*
Maturing at more than 150 cm	*Hemipristis elongatus* *Negaprion acutidens* *Galeocerdo cuvieri*

Among the four smaller species, both *Rhizoprionodon acutus* and *Loxodon macrorhinus* are active pelagic sharks with oblique, single cusps to the teeth of both jaws. Both species are widespread along the east coast of Africa but *R. acutus*, a common shark of the Natal coasts, is apparently more tolerant of dirty water than is *L. macrorhinus*, which is confined to the clear water to the north of St. Lucia. *Hypogaleus hyugaensis* is a poorly known species similar in morphology to the rather sluggish *Galeorhinus galeus*. It is found occasionally in Natal waters as far south as Durban. *Triaenodon obesus* is another tropical shark apparently confined to clear water, for it is fairly common on the Tongaland coast but has never been recorded south of St. Lucia. A tough-skinned and slow-swimming but agile shark, it is normally found near reefs where it feeds chiefly on crustaceans.

Of the three large species, only *Galeocerdo cuvieri* occurs regularly south of St. Lucia. A notorious scavenger with an appetite for anything from tin cans to human beings, it breeds in tropical waters with a few immatures and nonbreeding adults coming south into Natal and occasionally into eastern Cape waters. *Negaprion acutidens* appears to be confined to the clear waters of Tongaland and northward while little is known about the distribution of *Hemipristis elongatus*. Apparently it is fairly common in the northern Indian Ocean; specimens have been recorded from East Africa and from northern Mozambique, and a lone straggler was caught in a shark net on the southern Natal coast.

The three sphyrnid sharks found in the southwest Indian Ocean are members of the genus *Sphyrna*. The largest species, *S. mokarran*, is a widespread tropical species found as far south as the Natal coast where it occurs regularly but not commonly. All reproductive activities, including the nursery areas, seem confined to tropical waters. A similar situation exists for *S. lewini* except that a few newborn young have been taken off Natal and large numbers of juvenile (but not newborn) sharks are found in the area during the summer. *S. zygaena*, by contrast, has a distribution centered along the southern coast of South Africa and is seldom caught off Natal where occasional specimens are taken during the winter months.

Pseudotriakis and "Lower" Carcharhinids—*Pseudotriakis microdon* is a large (3 m) deepwater shark that probably occurs off the east coast of southern Africa but has not yet been definitely recorded there (specimens have been

taken northwest of Madagascar (Forster et al. 1970). Among the inshore species in this group are *Scylliogaleus quecketti, Triakis megalopterus*, and at least two species of *Mustelus*. These are demersal sharks feeding chiefly on crustaceans and attaining maximum lengths of 100 to 150 cm. *S. quecketti* is endemic to the Natal and eastern Cape coasts, while *T. megalopterus* is a temperate species ranging from the southwestern to the eastern Cape and southern Natal. *Mustelus palumbes* has a similar distribution but is a slighter if not shorter shark than *T. megalopterus*, while another species of *Mustelus* is found in shallow water off Natal and in deeper water on the continental shelf off southern Mozambique. A futher small species in this group, *Eridacnis sinuans*, grows to a length of about 40 cm. A demersal species, it has only been recorded from the Natal and southern Mozambique coasts at depths of 230 to 480 cm.

Scyliorhinids—The catsharks (family Scyliorhinidae) are mostly small (less than 1 m), sluggish sharks, most with restricted ranges and a consequent high degree of endemism. Bass et al. (1975*a*) recorded six genera from the east coast of southern Africa (excluding the deepwater genus *Apristurus*). Most are temperate species, those occurring in warmer areas being found chiefly on the continental shelf rather than close inshore. The ranges of the different species are briefly described as follows.

Genus *Haploblepharus*
    H. pictus. Southwestern Cape, in shallow water.
    H. edwardsii. In shallow water in the southern Cape; trawled on the continental shelf in the eastern Cape. Two specimens, possibly not of the same species, have been caught in shallow water in northern Natal.
    H. fuscus. In shallow water from the southern Cape to southern Natal.

Genus *Halaelurus*
    H. natalensis. In shallow water in the southern and southwestern Cape; trawled on the continental shelf in the eastern Cape.
    H. lineatus. In shallow water on the central Natal coast; trawled on the continental shelf off southern Mozambique.
    H. lutarius. In deep water on the continental shelf from southern Mozambique and Somalia.

Genus *Scyliorhinus*
    S. capensis. In shallow water in the southwestern and southern Cape; trawled on the continental shelf in the eastern Cape; taken once off central Natal in deep water near the top of the continental slope.

Genus *Poroderma*
    P. africanum. In shallow water in the southwestern Cape; taken by trawlers in the eastern Cape.
    P. pantherinum. In shallow water from the southwestern Cape to southern Natal, specimens from each end of the range are distinct from one another.
    P. marleyi. A rare species, known only from four specimens from the Natal coast.

Genus *Holohalaelurus*

H. regani. Taken on the continental shelf in the southwestern Cape; in Natal on the upper part of the continental slope, as in Mozambique; found at least as far north as Zanzibar. Specimens from the southwest Cape and Natal are distinguishable from each other.

H. punctatus. Rare in the southern Cape; common on the upper continental slope off Natal and Mozambique. Specimens from East Africa may be conspecific or a related species.

Genus *Cephaloscyllium*

C. sufflans. Taken in deep trawls off Natal and southern Mozambique coast. Specimens from Gulf of Aden may be another species.

Other Galeoid Sharks

Apart from the carcharhiniform sharks, the galeoid sharks include a number of other species which can be put into two groups: the orectolobiform and the lamniform sharks (after Compagno 1973).

Orectolobiform Sharks—The bulk of these sharks can be considered as the tropical equivalent of the shallow-water scyliorhinids of more temperate regions. Three species fitting in this category occur on the east coast of southern Africa. Only one of these, *Stegostoma varium*, extends into Natal waters; it is not uncommon in central Natal during the summer months. A sluggish shark attaining about 250 cm in length, it is primarily a mollusk eater although, like most sharks, it readily takes to a diet of fish. A smaller shark, attaining only about 60 cm in length, is *Ginglymostoma brevicaudatum*, as yet recorded only from shallow water in East Africa. Bearing a remarkable external resemblance to *Haploblepharus fuscus* of the Natal and southeastern Cape coasts, *G. brevicaudatum* may occupy a similar niche in its more tropical habitat. *Nebrius concolor*, a large (3 m), sluggish shark feeding chiefly on crustaceans and cephalopods, is found as far south as the Tongaland coast.

The remaining orectolobiform shark is the whale shark, *Rhiniodon typus.* Well known as the largest of modern fish, attaining a length of about 12 m and feeding by filtration through the gills, *R. typus* is a tropical species. Quite common along the eastern coast of Africa as far south as Tongaland, specimens are regularly seen off central Natal and occasional sightings are made in the eastern Cape and even in the southwestern Cape.

Lamniform Sharks—The lamniform sharks occurring regularly off the east coast of southern Africa include three species of *Odontaspis, Carcharodon carcharias, Isurus oxyrinchus,* and three species of *Alopias.* Little is known about the biology or distribution of the latter genus. *Alopias vulpinus* seems to occur regularly in the southern Cape while *A. pelagicus* and *A. superciliosus* have been recorded in central Natal and, in the case of *A. superciliosus,* in the southern Cape. *Carcharodon carcharias* was previously shown to be centered basically on the southern and southwestern Cape. *Isurus oxyrinchus* also seems to inhabit mainly temperate waters, though

confusion with the recently described *I. paucus* has resulted in uncertainty about the distribution of this genus in tropical seas. In the southwest Indian Ocean *I. oxyrinchus* certainly occurs regularly if not commonly on the Natal coast. Young specimens have been taken on numerous occasions along the eastern Cape and Natal coasts, which may be a nursery area for this shark.

In the genus *Odontaspis*, *O. taurus* has already been shown to be a shallow-water tropical species migrating southward to nursery grounds in the eastern and southern Cape. Two other species have also been recorded in the southwest Indian Ocean. *O. herbsti* is a large (up to 360 cm) inhabitant of the continental slopes, only occasionally coming into shallow water. A few juveniles have been trawled in Natal on the outer edges of the continental shelf, and Forster et al. (1970) took what appears to be a large female of this species in deep water to the northeast of Madagascar. The third species, *O. kamoharai* (often placed in a separate genus *Pseudocarcharias*), attains a length of about 100 cm and is usually found in warm open ocean waters. It is abundant in the Mozambique channel, and one sickly specimen was found swimming feebly in shallow water near Cape Town.

The Nongaleoid Sharks

The distribution of the nongaleoid sharks occurring off the east coast of southern Africa is briefly mentioned to complete the picture of the overall distribution of sharks in the region.

Heterodontidae—The one species found in the region, *Heterodontus ramalheira*, differs from most other heterodontids in that it is found on the continental shelf rather than close inshore. It has been recorded from Mozambique to the Arabian Peninsula.

Chlamydoselachidae—The only extant species, *Chlamydoselachus anguineus*, has been taken off the southwest African coast and possibly occurs in deep water off the east coast, where there has been one doubtful record (J. L. B. Smith 1951).

Hexanchidae—Four species have been recorded from the east coast of southern Africa (Bass et al. 1975d). *Notorynchus cepedianus* is a relatively large (up to almost 3 m) shark found in shallow water in temperate areas. It is fairly common on the Cape coast but rarely if ever ranges as far north as Natal. The other three sharks inhabit the continental shelves. *Heptranchias perlo* is a small species, growing to about 135 cm, with a tropical distribution. It is fairly common off southern Mozambique, and two specimens have been caught off central Natal while none has been recorded from further south. There are two species of *Hexanchus*, *H. vitulus* and *H. griseus*. *H. griseus* is a large shark, growing to nearly 5 m, while *H. vitulus* is mature at 120 to 140 cm and grows to no more than about 180 cm. A tropical species, it is a common shark from East Africa to southern Mozambique and two specimens have been taken in central Natal. In temperate waters this shark

appears to be replaced by juvenile *H. griseus*. Adult *H. griseus* are usually found in tropical waters.

Squatinidae—*Squatina africana*, the only representative of this group in the southwest Indian Ocean, grows to slightly more than a meter in length and is found in shallow water and on the continental shelf from southern Mozambique to the eastern Cape. It is adapted to living on the seabed, and its ecological position is among the batoid fishes rather than the sharks.

Pristiophoridae—The sole representative of this group in the region is *Pliotrema warreni*, a small (up to 136 cm) sawshark found on the continental shelf from the southern Cape to central Natal and occasionally in the south-western Cape and in southern Mozambique. This shark also belongs among the batoid fishes in any ecological classification.

Oxynotidae—No members of this group have been found on the east coast, the nearest records being two *Oxynotus* from the southwestern Cape.

Dalatiidae—This family of squaloid sharks is represented off the east coast of southern Africa by a continental shelf species and at least two dwarf pelagic species living in deep water. The latter include *Euprotomicrus bispinatus*, a well-known shark with an antitropical distribution, and *Heteroscymnoides marleyi*, known only from a single juvenile washed up on a Durban beach. The tropical pelagic delatiid *Isistius brasiliensis* may yet be found in the Mozambique channel. The continental shelf species is *Dalatias licha*, a sluggish shark of medium size ranging northward from the southern Cape to at least southern Mozambique.

Echinorhinidae—The medium-sized *Echinorhinus brucus* is fairly common in the continental shelf areas of the Cape coasts but is rare in Natal, where occasional specimens are caught in the southern areas.

Squalidae—The taxonomy of the squalid sharks is still rather con-fused, and it would serve no purpose here to consider their distribution in any detail. Suffice it to say that they are basically small and medium-sized demersal sharks competing to some extent with the "lower" carcharhinids, from which they differ in the possession of cutting rather than grasping or crushing teeth. Found on the continental shelves and in deeper water in temperate and tropical areas, many of the species have wide distributions. The squalids are among the most numerous of sharks, both in actual numbers and in numbers of species.

INTERACTIONS BETWEEN SPECIES

In an article entitled "Social Organization of Shark Populations" Stewart Springer (1967) gave an excellent analysis of the ways in which the distribu-tion of sharks can be affected by inter- and intraspecific reactions. Apart from emphasizing the need for consideration of size and sexual segregation when describing the distribution of any species, he put forward the idea that

"The availability of nursery areas, not only suitable to each species but also comparatively free of predation by larger sharks, is very important in inter-species competition."

The data gathered on shark distribution off the east coast of southern Africa corroborate this view. It is apparent that not only the large species exhibit marked segregation (see also, for instance, the study of the small *Galeus arae* by Bullis (1967). In many cases the apparent homogeneity of the populations of small species is due to sampling on too large a scale. Springer concludes that sharks "usually have little difficulty in finding enough food, despite annual or seasonal fluctuations in the supply. . . . The implication must follow that some factors other than food supply hold populations of sharks at some point below the level of the maximum popula-tion density that could be sustained by the available food supply." He also notes that the availability of suitable nursery grounds might be a factor limiting the total population of a system. I fully agree with this reasoning but would like to approach the subject from a different viewpoint—that not only the numbers of individuals in a given population are affected in this way but also the geographic range inhabited by the species.

A first example is the sharks of the family Hexanchidae as represented in the southwest Indian Ocean. The only inshore species, *Notorynchus cepedianus*, will not be discussed here. Three further species are found on the continental shelf. The small shark *Heptranchias perlo* is found chiefly in tropical areas. In much the same habitat (as far as we know) lives *Hexanchus vitulus*, a slightly larger shark growing to a length of about 180 cm. *Hexanchus griseus*, by contrast, grows to more than 4 m. The adults of this large species occur chiefly in tropical waters, but young specimens of a size equivalent to that of mature *H. vitulus* appear to be restricted to the cooler areas of Natal and the eastern Cape. It does not seem unreasonable to suggest that *H. griseus* has a nursery area in a temperate region because of the com-petition from adult *H. vitulus*, which inhabits the same latitudes as the adult *H. griseus*. It could be an adaptation against predation by large *H. griseus* on their own young, but, if so, it is difficult to understand how *H. vitulus* manages to survive. Predation by large sharks may be less important in this case than competition among equivalent-sized animals of different species.

In the case of *Hexanchus griseus*, it seems that the nursery areas are in temperate regions fringing the tropical habitat of the adults. The range of any self-perpetuating population of *H. griseus* adults must therefore be restricted to migratory distance from a suitable temperate nursery area. The northward spread of *H. griseus* in the Indian Ocean may well be restrained (at least in the western regions) by the lack of subtropical continental shelf nursery areas for the young except off the southeast coasts of southern Africa. An analogous situation occurs in *Odontaspis taurus*, in which the adults of both sexes are found in clear, tropical inshore water off southern Mozambi-que and Tongaland. Pregnant females migrate south during winter to drop their young in the nursery grounds off the eastern and southern Cape. The northern limits of *O. taurus* along the east African coast have not been defined; it is unknown from Kenya, Tanzania, and Madagascar. Madagascar

would seem to be an ideal habitat for *O. taurus* and probably is, except for the lack of suitable nursery grounds within migratory distance. As noted by Bass et al. (1975c), "the Mozambique channel may possibly be crossed by an occasional vagrant *O. tarus*, but this wide expanse of deep water presents a real obstacle to regular migrations by any species which normally lives on the continental shelf."

A further point arising from this train of thought is whether sharks stay in a particular habitat because they have to (for some physiological reason) or because they have no particular reason to move out of it. A consideration of the sharks *Triaenodon obesus*, *Negaprion acutidens*, and *Odontaspis taurus* can clarify this rather obscure statement. Both *T. obesus* and *N. acutidens* occur in shallow water off Mozambique and Tongaland but are rarely, if ever, found in the turbid and slightly cooler waters to the south of St. Lucia. Adult *O. taurus* have the same basic distribution, except that pregnant females swim southward to the nursery areas in the southern and eastern Cape. Both *T. obesus* and *N. acutidens* complete their life cycles in tropical waters and therefore have no need to move into subtropical regions. If *O. taurus* had nursery areas in tropical rather than subtropical waters it would also appear to be confined to tropical seas. That certain sharks do not normally inhabit cold, warm, clear, turbid, oxygen-deficient, or brack waters may reflect a preference rather than a physiological inability to tolerate such conditions.

Carcharhinus leucas is well known for its ability to enter freshwater and uses rivers and lake systems along the east African coast as nursery areas. The young sharks also occur in the sea and are quite capable of living in normal marine salinities. If this species did not use rivers as nursery areas (remembering that the adult sharks do not normally enter freshwater in the region) would anyone have suspected that it was capable of osmoregulatng in freshwater? *Carcharhinus amboinensis*, a species very similar to *C. leucas* in morphology, is common on the Natal coast (more so than *C. leucas*) but has never been recorded in any of the river systems along the east African coast. The logical explanation of these distributions would be that *C. leucas* and *C. amboinensis* avoid competition by having different nursery areas, *C. leucas* in freshwater and *C. amboinensis* in the sea.

At Madagascar, however, *C. leucas* appears to be absent and *C. amboinensis* is found close inshore and possibly also in freshwater. This shark has been reported from freshwater bays along the New Guinea coast where strong river outflows displace the seawater, but it is not known whether it actually ascends the river systems (L. F. Filewood, personal communication). *C. leucas* and *C. amboinensis* may avoid competition by having nursery grounds in different regions, both of them in freshwater. In the southwest Indian Ocean, *C. leucas* uses river systems along the African coast while *C. amboinensis* may possibly use those of Madagascar and other islands. Research into the habits of these two species in strictly tropical regions should yield some interesting results. One can also speculate as to how many sharks not normally found in freshwater are physiologically capable of adjusting to such an environment.

An interesting feature of the distribution of nursery areas along the east coast of southern Africa is that secondary nursery areas of tropical species often extend further into subtropical regions than do the primary nursery areas. By primary nursery areas is meant those where the young sharks are actually born and spend the first part of their lives. Secondary areas are those inhabited by the slightly older but not yet adolescent or mature sharks.

An example of this phenomenon is shown by *Carcharhinus plumbeus*. Immature *C. plumbeus* of 90 to 160 cm are fairly common in Natal waters, but newborn specimens (60 to 65 cm in length) are completely absent. The only record in this size range comes from a depth of 280 m off southern Mozambique. The few records of adult *C. plumbeus* from Natal are mostly of pregnant females with full-term embryos (Bass et al. 1973). It would seem that the primary nursery area is off southern Mozambique and that the young sharks move southward into Natal waters when they attain a length of about 90 cm. *Carcharhinus obscurus* shows a similar pattern with the primary nursery area on the southern Natal coast and a secondary area ranging from northern Natal to the southern Cape. This general pattern of having secondary nursery areas in cooler areas than primary nurseries is also seen in *Carcharhinus amboinensis*, *Carcharhinus limbatus*, *Carcharhinus brevipinna*, *Carcharhinus altimus*, and *Sphyrna lewini*. One reason for the evolution of such a pattern could be that the adults do not then have to make extensive migrations into unfamiliar and possibly unsuitable environments which can still be used as nursery areas by their young.

Reproductive migrations in small sharks (maximum length less than 150 cm) generally seem to involve movements from one depth to another without any large-scale geographic movements. The nursery areas of such species are usually close to the adult habitats but at markedly different depths. Examples include *Galeus arae*—the adults live in greater depths than the juveniles (Bullis 1967)—and *Holohalaelurus regani*—the nursery areas are in deeper water than that normally occupied by the adults (Bass et al. 1975a). In contrast to the restraints on the range of a species such as *Odontaspis taurus* caused by the use of distant nursery areas, small sharks are able to use suitable habitats to the full.

This is particularly noticeable in the case of oceanic islands, of which Tromelin Island in the western Indian Ocean is a good example. An isolated oceanic island to the east of Madagascar (Figure 1), Tromelin is over 200 n.mi. from any other piece of land or continental shelf. Despite this the island has a flourishing population of *Triaenodon obesus*, a sluggish shark normally found in shallow water in the vicinity of reefs but nevertheless widely distributed over most of the Indian and western Pacific oceans. Only a species having a concise distribution during its life cycle would be able to survive around most small oceanic islands.

Recognition Among Sharks

Segregation by size and sex is virtually universal among sharks as is the use of distinct nursery areas, usually at different depths from those inhabited by

the adults and often in geographically distant localities. It is now apparent that such segregation is not confined to the larger species but is present even in sharks as small as *Galeus arae* (maturing at 25 to 28 cm (Bullis 1967) and *Holohalaelurus punctatus* (maturing at 24 to 29 cm (Bass et al. 1975a)). While predation of small sharks by large sharks is one factor influencing the evolution of distinct nursery areas, it is possibly less important than competition between equivalent-sized animals of different species. Food does not seem to be the limiting factor; space or the lack of it must be taken into consideration. The work of Johnson and Nelson (1973) on agonistic display in *Carcharhinus menisorrah* (=*C. amblyrhynchos*) was the first systematic investigation of territorial behavior in sharks. These authors quote several other references to territoriality and to interspecific dominance in sharks, of which the observations of Limbaugh (1963) are particularly relevant to this discussion. Writing about the behavior of three species of *Carcharhinus* found near Clipperton Island in the eastern Pacific, he noted that there was "a definite nip order among these three species of sharks. The larger sharks within a species dominate the smaller ones. Among the sharks of nearly the same length, the whitetip reef shark (*C. platyrhynchus*, ?=*C. albimarginatus*) very clearly dominated. The Galapagos shark (*C. galapagensis*) dominated the blacktip shark (*C. limbatus*), which seemed almost afraid of its own shadow."

Further work will probably emphasize that territorial behavior is widespread among sharks. This sort of behavior would probably not have evolved unless populations tended to increase in size until regulation was necessary in terms of space. In the presence of adequate food supplies, territorial considerations would seem to be the principal influence on the distribution of the various age and sex groups of any shark species. The study of social behavior in sharks is thus likely to become increasingly relevant to studies of their ecology and population dynamics. One topic that deserves more attention is recognition among sharks. This includes recognition not only of different species but also of sex, size, and physiological condition in the same species. Adult sharks of different species but similar size may inhabit the same area. How do they recognize each other, and what factors ensure that females are fertilized by males of their own species? As sharks are not known to make any sounds it seems unlikely that they communicate in this way, and recognition is probably made by means of visual or chemical signals. Visual signals can be divided into two arbitrary groups for descriptive purposes—locomotory and postural displays and distinctive markings on the body and fins.

Locomotory and Postural Displays—The best description of this type of display is that by Johnson and Nelson (1973) of agonistic display in *Carcharhinus menisorrah* (=*C. amblyrhynchos*). This consisted of a distinctive posture as well as laterally exaggerated swimming and rolling or looping. The authors felt this display expressed defensive threat. "It appeared ritualized in nature and is likely to be of value in normal social encounters."

Courtship display, in which males make surface bites on the pelvic areas as well as other parts of the body and fins of females, also fits into this

category. Although visible evidence of this habit is often seen in the form of mating scars on females and in sexual dimorphism in tooth shape, the only description of this type of behavior is that given by Clark (1975) for a species of *Carcharhinus* (either *C. spallanzani* or *C. amblyrhynchos*) in the Red Sea: ". . . earlier we had seen 15 sharks of this genus, *Carcharhinus*, in a courtship frenzy. They were milling around in a loose group. Then a female broke away from the throng and swam upward with a male following and biting her repeatedly, tearing the edges of her fins, slashing the sides of her body, leaving a crescent of tooth marks on her flanks."

Distinctive Markings—Distinctive markings on the body and fins might be used in conjunction with postural displays to show them off to better advantage or might serve as an efficient means of communication between sharks swimming in a relaxed "normal" manner. The markings found on many sluggish, bottom-dwelling sharks serve to camouflage them rather than to make them more conspicuous, and it is among the pelagic species that distinctive markings are chiefly found. In the deeper parts of the oceans many shark species have complex patterns of photophores that may be of use in maintaining the integrity of schools of the same species (Springer 1967).

Variations among distinctive markings can be seen in the genus *Carcharhinus* as recorded off the east coast of southern Africa. Thirteen species with tropical distributions are found in inshore waters of this region, and these can be divided into two groups on the basis of the presence or absence of distinctive markings on the adult sharks.

Species without distinctive markings—Six species occur: *C. leucas*, *C. amboinensis*, *C. obscurus*, *C. falciformis*, *C. altimus*, and *C. plumbeus*. The first five tend to have dusky tips to the fins in juveniles which fade and may be imperceptible in the adults. *C. plumbeus* is completely plain from birth to adulthood. *C. obscurus* and *C. falciformis* are inhabitants of the outer parts of the continental shelf and possibly do not come into regular social contact with other species of their genus. The same can be supposed for *C. altimus*, which is normally found near the bottom on the continental shelf.

Species with distinctive markings—Seven species occur here, *C. brevipinna*, *C. limbatus*, *C. spallanzani*, *C. sorrah*, *C. melanopterus*, *C. albimarginatus*, and *C. sealei*. The distinctive markings of these species may be present from birth as in *C. albimarginatus* and *C. sorrah* or may appear some time after birth as in *C. brevipinna*, the newborn young of which have no markings at all. On the whole, these are inshore sharks commonly found in clear water and visual distinctions might help considerably in specific recognition. *C. plumbeus* is also commonly found with these species but might be distinctive in its lack of markings. In the Mauritius-Seychelles area the pale color of this shark is responsible for its vernacular name of "requin blanc." *C. leucas* and *C. amboinensis* may occur in the same waters as these sharks. Although distinctive markings are probably not the only method by which these various species recognize one another, they are probably helpful

in a great number of cases. The best evidence of this is given in Figure 14 which shows lateral views of several of these sharks. In combination with size, habitat, and behavioral differences the distinctive markings of many of these animals probably result in efficient recognition of at least their own species.

Apart from distinctive markings visible from a lateral view, a ventral view of many sharks reveals distinctive black tips to the pectoral and/or pelvic fins. These can be seen in sharks from diverse groups and habitats such as *Carcharhinus longimanus, C. brevipinna, C. limbatus* (Figure 15) and *Carcharodon carcharias* (Figure 8). Noteworthy here is the difference between the pelvic fin markings of *Carcharhinus brevipinna* and *C. limbatus. C. limbatus* has distinct black tips to the pelvic fins of adults and all the other fins have rather blurred dark tips. In *C. brevipinna*, a species growing to a similar size and occupying much the same ecological niche as *C. limbatus*, all the fins have clearly demarcated black tips except for the pelvic fins, which are completely plain.

Chemical Signals—I do not know of any references to the release of pheromones by sharks but consider it highly likely to occur, particularly by females ready for mating. Attraction of a mate of the correct species could be by postural or locomotory displays, but it seems far more likely that animals with as highly developed an olfactory system as sharks would at least initiate courtship by means of a chemical signal.

Knowledge of the ways in which sharks recognize one another is sparse, to say the least. Distinctive markings and postures, locomotory displays, and chemical signaling probably have some part to play, and further work along these lines will not only increase our knowledge of the sensory physiology of sharks but also help us to understand the ecology and population dynamics of these fascinating but poorly known animals.

(A) A 121-cm mature male *C. spallanzani*

Figure 14 Some of the *Carcharhinus* species found in the tropical parts of the southwest Indian Ocean, showing distinctive patterns of markings. (After Figs. 6, 20, 26, 28, 29, and 31 of Bass et al. 1973.)
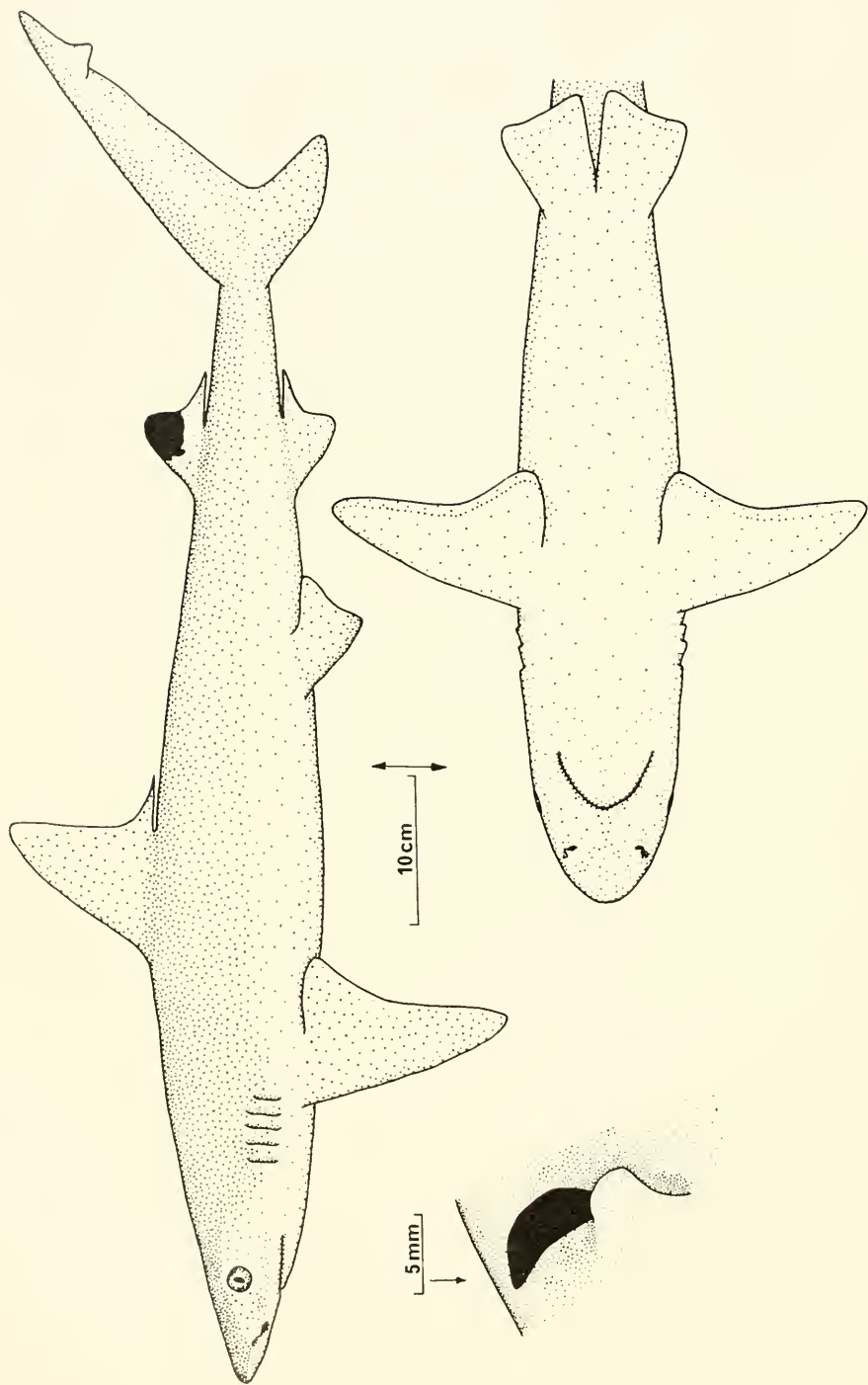
(B) A 91-cm mature female C. *sealei*

10 cm

5 mm
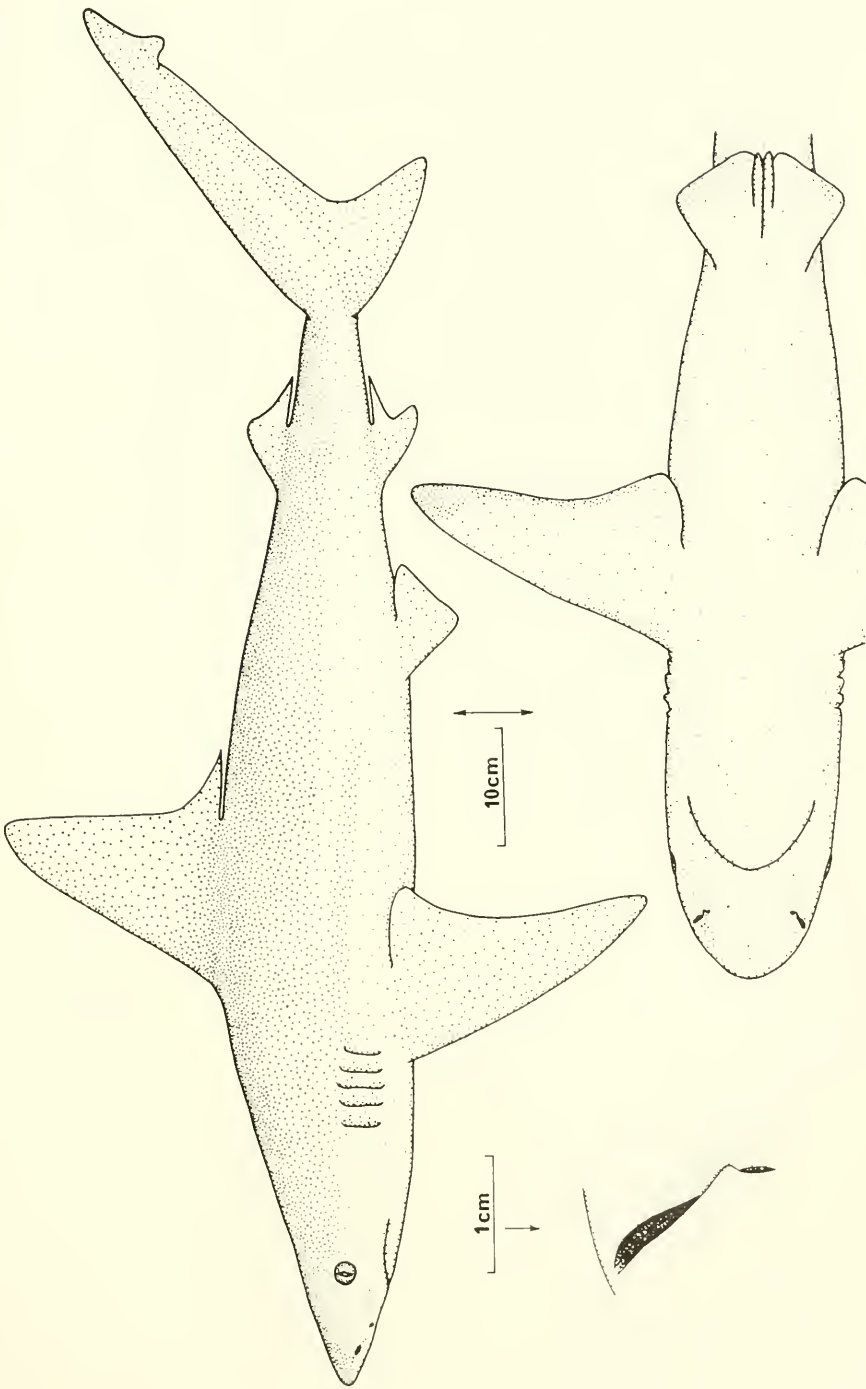
(C) A 118-cm immature male *C. plumbeus*

Figure 14 Some of the *Carcharhinus* species found in the tropical parts of the southwest Indian Ocean, showing distinctive patterns of markings. (After Figs. 6, 20, 26, 28, 29, and 31 of Bass et al. 1973.)
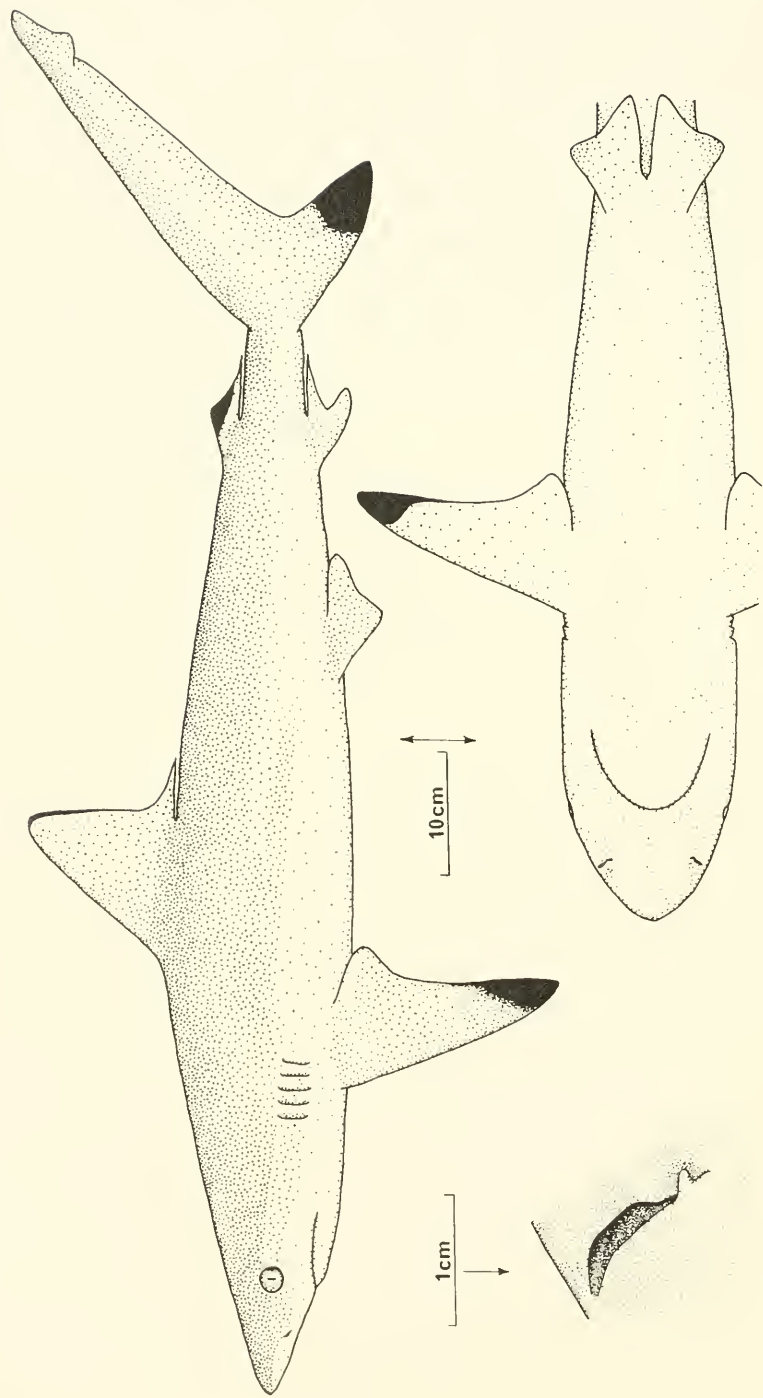
(D) A 111-cm mature female *C. sorrah*

10cm

1cm
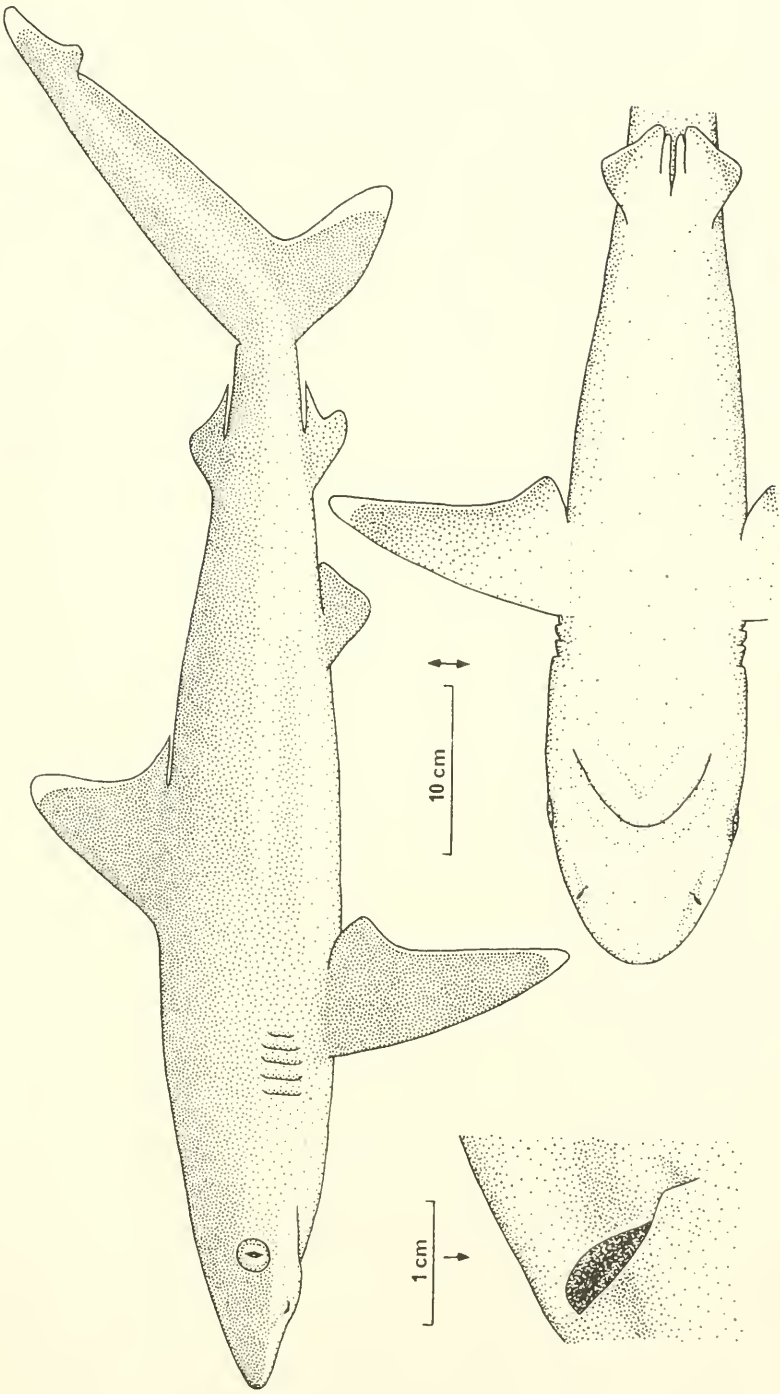
(E) An 83-cm immature male *C. albimarginatus*

Figure 14 Some of the *Carcharhinus* species found in the tropical parts of the southwest Indian Ocean, showing distinctive patterns of markings. (After Figs. 6, 20, 26, 28, 29, and 31 of Bass et al. 1973.)

(F) A 70-cm immature male C. *melanopterus*

Figure 14 Some of the *Carcharhinus* species found in the tropical parts of the southwest Indian Ocean, showing distinctive patterns of markings. (After Figs. 6, 20, 26, 28, 29, and 31 of Bass et al. 1973.)

10cm

1cm
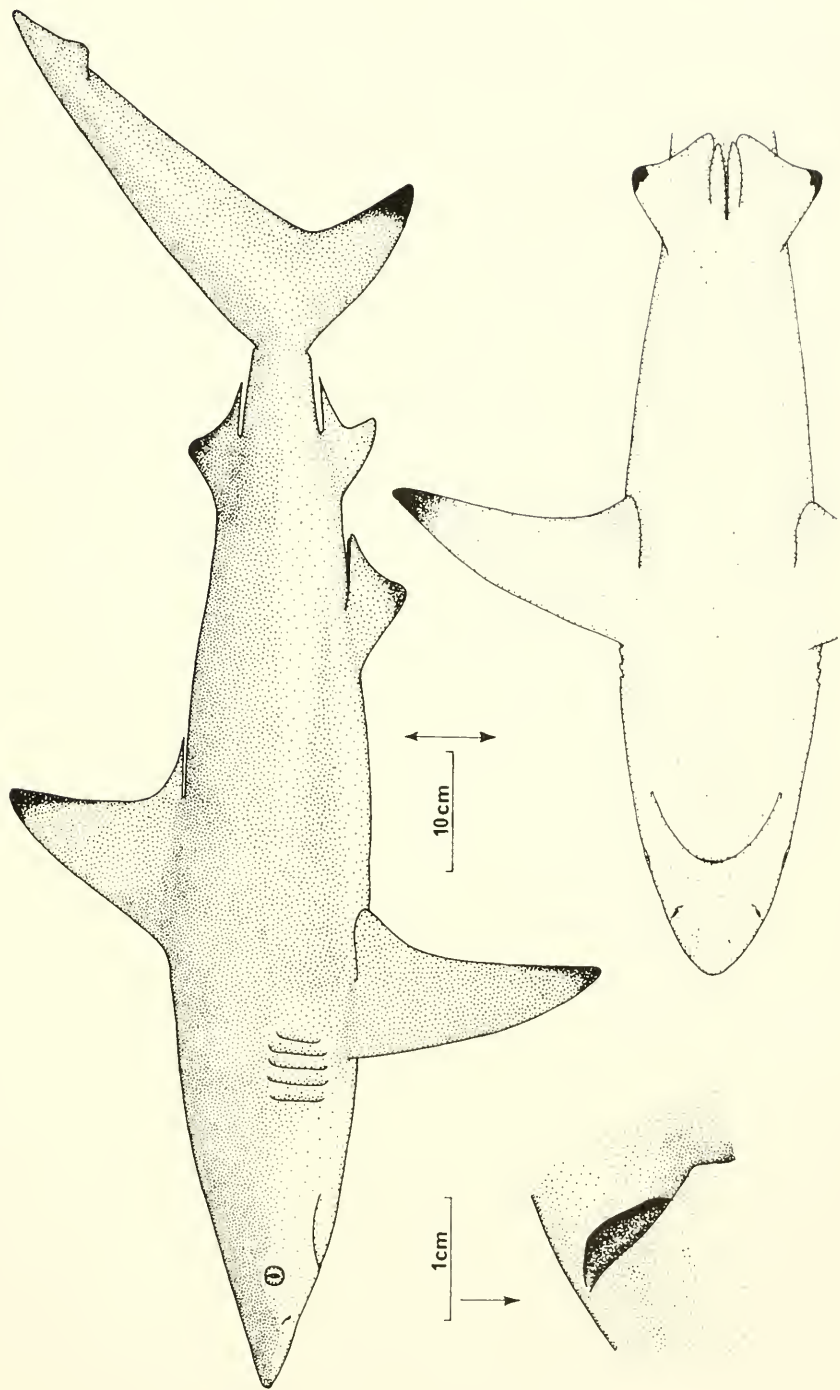
(A) A 115-cm immature male *Carcharhinus limbatus*

Figure 15 Ventral views of some sharks from the southwest Indian Ocean, showing distinctive patterns of markings. See also Figure 8. (After Figs. 9, 17, and 19 of Bass et al. 1973.)
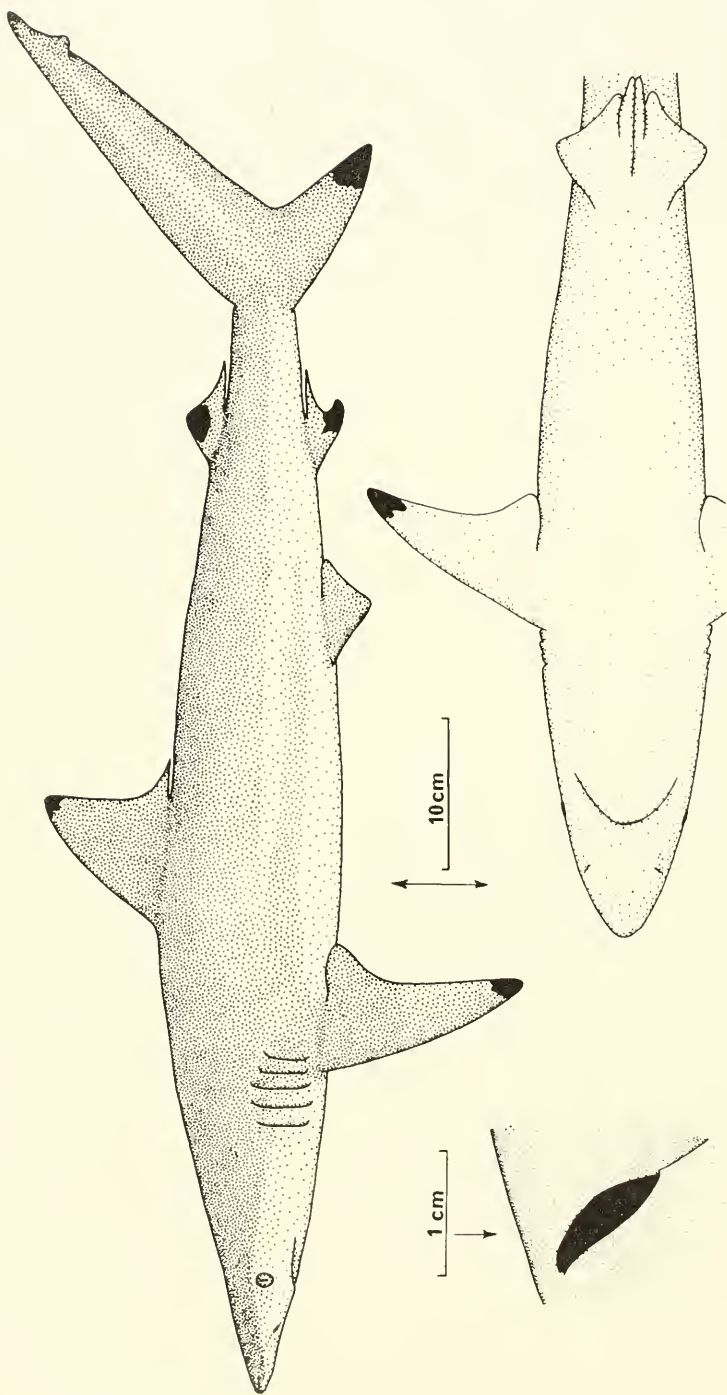
(B) A 179-cm adolescent male *Carcharhinus brevipinna*

Figure 15   Ventral views of some sharks from the southwest Indian Ocean, showing distinctive patterns of markings. See also Figure 8. (After Figs. 9, 17, and 19 of Bass et al. 1973.)
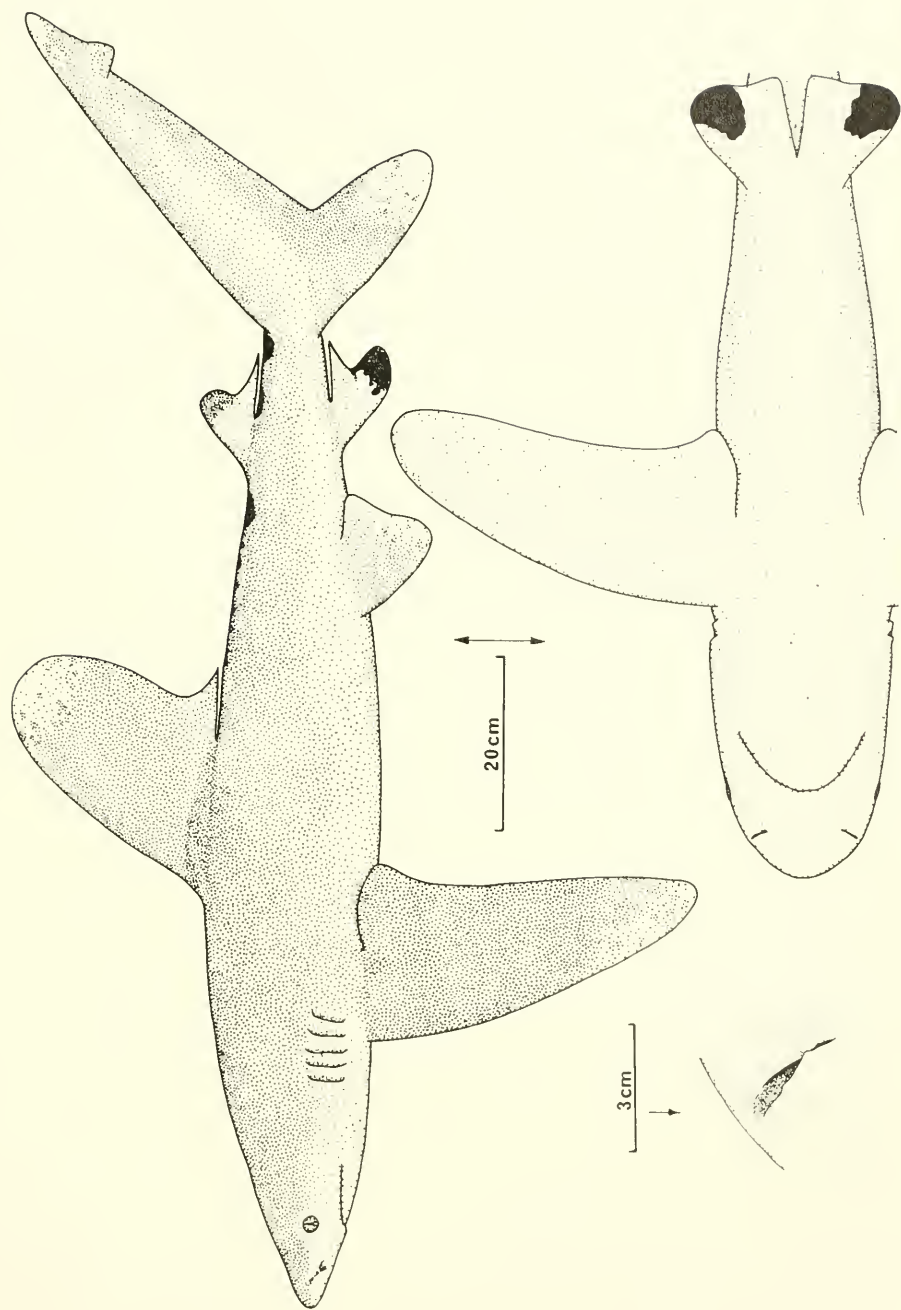
(C) A 150-cm immature female *Carcharhinus longimanus*

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KNOWLEDGE AND EXPLOITATION OF THE
SENSORY BIOLOGY OF SHARKS IN THE SOUTHWESTERN PACIFIC

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Author (right) discussing uses of elasmobranch models from Fais with Mr. Raphael Uag, Yap Museum, western Caroline Islands.

INTRODUCTION

No region on earth exceeds, or in all likelihood equals, the southwestern Pacific Ocean in the frequency and variety of encounters between men and sharks. Throughout an area of more than 15 million square miles, geological and ecological evolution have produced environments that sustain large and diverse shark populations. Certain species of sharks are everyday components in the lives of island and coastal peoples of this vast area.

In the southwestern Pacific, sharks are important, even preferred, food sources. They also provide valuable materials for tools or artifacts serving as media of exchange. Not surprisingly, a preoccupation with the habits and characteristics of various sharks is evidenced by their incorporation into artistic motifs and religious rituals in many localities. Elaborate and careful training may be given to children, especially boys, in the methods of detecting, capturing, safe handling, and otherwise exploiting sharks.

Even a cursory acquaintance with the indigenous cultures of Micronesia, the Gilbert Islands, or the Solomon Islands (to name only a few) reveals abundant folk knowledge about sharks. It is easy to overlook or dismiss such knowledge as simple, primitive, and essentially valueless for serious study, but this is a mistake. Unfortunately, it may account in part for the scant attention paid to such matters in the past, and for certain inadequacies of even the most detailed ethnographic surveys.

To consider the facts from a very different viewpoint, one need only reflect that sharks are about as commonplace to many Pacific islanders as automobiles are in urban Western cultures. It would be surprising, therefore, if local knowledge from the Pacific failed to provide clues to aspects of shark biology worthy of further investigation through controlled laboratory and physiological techniques. Since the eventual applications of many laboratory studies on sharks might be to increase the predictability and safety of man-shark interactions in natural situations, it seems appropriate to examine the procedures developed toward that same end by many generations of Pacific islanders. They have worked out their procedures by trial and error, under conditions that certainly include strong selective factors against mistakes!

The object of the present analysis is twofold: first, to compare some of the knowledge obtained from modern scientific studies on the sensory biology of sharks with the empirical knowledge included in certain Pacific cultures, and second, to discover any local observations from the southwestern Pacific suggestive of additional problems worthwhile for scientific study.

At the outset, it must be frankly admitted that the analysis is of necessity highly selective and incomplete. Published anthropological reports on shark fishing, for example, while extremely valuable, typically have not been assembled in cognizance of modern knowledge about the biology of sharks. Hence, they are often inadequate for the present considerations. Cross-checking information, much of it necessarily available only from verbal accounts, is time consuming but essential; this too imposes an important limitation on the scope of the present study. One of the values of these

observations may be to bring into focus a rewarding area for further combined biological and anthropological research.

LOCALITIES AND SOURCES

Field observations and interviews were carried out between November 1974 and March 1975, in Micronesia and northeastern Australia. Yap, in the western Caroline Islands of Micronesia, yielded especially interesting information. The extensive collections of the Micronesian Area Research Center (MARC) at the University of Guam were particularly helpful and were used extensively. Ethnographic materials from the Solomon Islands and the Northern Territory of Australia were studied at the Museum of the Northern Territory (Darwin) and the Australian National Museum (Sydney), and later in the British Museum (London). Access to museum materials and invaluable discussions of their uses and interpretations resulted from the generous aid of specialists at each of these institutions.

Information concerning the Fiji and Gilbert Island groups was drawn mainly from interviews with Dr. Ronald Gatty, whose personal experiences with sharks and shark fishermen in those areas is probably unexcelled by any other Western observer. In addition, Dr. Gatty provided translations and a manuscript copy of his major study on Fiji, now in preparation (Gatty 1978).

To overcome problems of misunderstandings or inaccuracies in the verbal source material (never previously recorded so far as is known), we held interviews with at least three individuals who could provide first-hand accounts of local observations on sharks. No information not consistently reported by all three sources has been incorporated in this summary, unless it could be checked by direct personal observation. No doubt some valuable material has been excluded by this conservative policy, but it was judged better to provide a firm basis for future studies rather than strive for premature, and possibly erroneous, comprehensiveness.

INSIGHTS AND EXPLOITATIONS OF SENSORY MODALITIES

In the southwestern Pacific, knowledge of the sensory abilities and behavior of sharks is used most often in fishing. It may also be important in connection with safety precautions taken by those active in waters frequented by potentially dangerous sharks. Parallels between scientific conclusions and strictly local empirical observations in the Pacific area are sometimes quite striking. They are most conveniently discussed according to the sensory modalities involved.

Acousticolateralis System

Attraction of Sharks by Sound—Nelson and Gruber (1963) and Nelson and Johnson (1972) demonstrated that pulsed low-frequency sounds attract many species of Atlantic and Pacific sharks. A comprehensive review

of the phenomenon is provided in this volume by Myrberg (1978). Pulse intermittency is especially important in eliciting oriented behavior of sharks, and an irregular series of pulse trains is more effective than a continuous output.

The use of sound to attract sharks is common among Pacific shark fishermen. It has been documented in the Society Islands (Gibbings 1948, Price 1944) Gilbert Islands (Anell 1955, Gatty 1978), Fiju (Gatty 1978), Caroline Islands (Price 1944) and several other island groups (Anell 1955).

The most commonly used sound sources are rattles made of coconut shells (Figure 1). Broken coconut shells may be strung on a twig bent into a circle, so that the rattle resembles a necklace. In the linear model of this same apparatus, the shells are strung on a rope made of plant fiber, and small cowrie shells may be attached along the rope to provide a more rapid, higher-pitched, jingling sound that contrasts with the low-frequency sound from the coconut shells.

The methods of using coconut rattles fall into at least two categories:

1. The rattles are vigorously worked up and down, churning the water surface into a foam. The sound is often claimed to resemble a shoal of small fish breaking on the surface (e.g., Gibbings 1948). In a demonstration of this
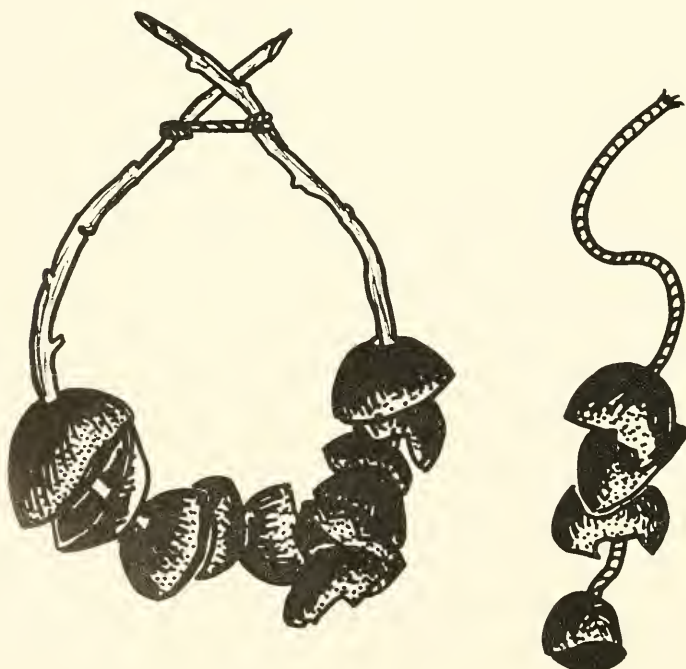
Figure 1   Shark rattle lures, constructed from coconut shells, Micronesia.

method in the western Caroline Islands, the surface was churned con-
tinuously for periods of 20 to 70 s, interrupted by approximately equal
periods of silence.

2. The coconut shells are shaken underwater, or scraped against the side
of the boat, producing intermittent bursts of low-frequency sound. Sound
output may be augmented by pounding on the hull or gunwales of the
fishing boat during the same intervals.

These methods of attracting sharks are reported effective with grey reef
sharks (*Carcharhinus menisorrah*), reef whitetips (*Triaenodon obesus*),
hammerheads (*Sphyrna sp.*), tiger sharks (*Galeocerdo cuvieri*), and makos
(*Isurus oxyrinchuss*); this list is doubtless incomplete. In Micronesia, the
Gilberts, and Fiji, at least, great emphasis is placed on the importance of the
"right rhythm," which includes not only the rhythm of shaking the rattles
underwater, but also the relation of periods of sound with intervals of quiet.
Variability of acoustic stimulation is the common denominator of all these
procedures, and it is quite likely that the trial-and-error approach has hit
upon the significant role of habituation in eliminating a shark's responses if
the stimulus is continuous.

A particularly dramatic method of varying the acoustic attraction of
sharks has been documented in several islands (Eua, Iki, Móungáone, and
Uiba) of the Tonga group, where shark fishing has become a highly ritualized
procedure. Use of the rattle may be alternated with intervals when one of
the fishermen jumps into the water himself, splashing about to "encourage"
the sharks (Gibbings 1948). When sharks are attracted to the general area but
are not close enough for noosing (the usual method of capture there), it is
not unusual for a swimmer to approach the sharks and then turn to lead
them to where they may be captured. This is locally interpreted as over-
coming the sharks' "shyness"—a deficiency from which the swimmers them-
selves evidently do not suffer!

The success of the island fishermen in attracting sharks with these pro-
cedures is all the more remarkable when it is remembered that the sound
sources are limited to the area next to a boat at the water surface, whereas in
the controlled studies of Nelson and Johnson (1972), at Eniwetok Atoll, the
amplified test sounds were played into the water from a transducer (speaker)
lowered within a few meters of the bottom or of coral reefs frequented by
sharks. Speedier arrivals at the sound source area would be expected under
the latter conditions and were, in fact, obtained (Nelson and Johnson 1972).
Nor can it be claimed that the optimal procedures, derived from local empir-
ical observations, are always used in Pacific fisheries. More experienced and
expert shark fishermen appear to use quite deliberate care in achieving just
the type of acoustic stimuli they judge effective; less experienced fishermen
may be more casual.

When patience is exhausted by prolonged lack of results, however, almost
any shark fishing expedition appears to turn to a "try anything" approach,
with splashing, pounding the boat hull, clattering of rattles and any other
noisemaking procedure. This is typically more of an emotional catharsis for

the fishermen than an added attraction for the sharks. A consideration that might be worthy of ethnographic study is the extent to which (if at all) the rituals of shark fishing serve to transmit and assure the most effective use of procedures developed over many generations. Such cultural traditions would obviously confer major benefits to the local society, but they have not yet been adequately studied.

Repulsion of Sharks by Sound—Although underwater sounds are used most commonly, by far, to *attract* sharks, there are a few instances and conditions in which sharks are reported to be *repelled* by sound. The evidence is fragmentary, but it is all the more interesting because experimental studies with controlled auditory stimuli have failed thus far to find a reproducible method for repelling sharks by sounds.

In Fiji, when netting fish ("busa") swimming near the surface, men dive around the net to watch it underwater. Quite commonly, there are sharks around them. (The shark species could not be identified from descriptions, but lengths of 4 or 5 ft are said to be most common.) The divers knock two oarlocks together underwater to scare the sharks away—a method said to be very effective.

This procedure has been used for a long time, in Fiji at least, for it is reported that in earlier times stones rather than oarlocks were used for the same purpose. The phrase "giria vata na roloka" ("ring together the stones") is used to describe the method (Gatty 1978).

Safety and Recognition of Shark Sounds—Safety precautions of Pacific islanders include obvious consideration of the effects of acoustic stimulation upon sharks. As noted above, fishermen use a variety of sounds, even the sound of their own swimming, to lure sharks closer. Divers, on the other hand, usually wish to avoid sharks, and hence try not to splash at the surface. For example, Gilbertese divers are trained to *slide* into the water, never to jump from their boats (Gatty 1978). Interestingly enough, despite the recognition of the sensitivity of sharks to surface splashes, this safety consideration has not been explicitly publicized among Western divers (e.g., Gilbert 1963).

One of the most remarkable achievements of divers in some parts of the southwestern Pacific is their recognition of certain species of sharks, and certain activity patterns of sharks, from underwater auditory cues. Expert divers among the Gilbert Islanders claim to recognize at least three different species of sharks from their characteristic swimming sounds. The tiger shark (*Galeocerdo cuvieri*) is considered "easiest" to recognize, but the identities of the other two were not clear from the descriptions. Gatty (1978), who has spent much time underwater with Gilbertese divers, confirms that the "throbbing" sound produced by tail motions of some large sharks can be differentiated from ambient hydrodynamic sounds, but that these distinctions were not clear to him until pointed out by the Gilbertese.

In the same area, divers report that they can recognize two activity patterns of large sharks by the characteristic swimming sounds. "Voyaging" swimming is differentiated from "cruising, investigating, or hunting." The

voyaging pattern involves rapid swimming and is not considered a threat to the divers. Swimming sounds produced by cruising are more irregular and, when detected, they are considered to indicate a threatening situation.

The Chemical Senses

The use of chemical stimulation from meat or blood of bait fish is so well known and universal in shark fishing throughout the Pacific that it needs no extensive discussion. Any small fish (flying fish, mackerel, etc.) or lobster is considered good bait. If the bait is more than about 300 mm (1 ft) long, it is usually broken into pieces of convenient size. The smallest pieces are used for chumming sharks alongside a boat for hooking, harpooning, or noosing. The use of fishhooks and harpoons is similar to procedures followed in other parts of the world, although the fishing gear is generally made of local materials. Noosing sharks, especially the larger specimens, is a widespread practice, and involves a high degree of skill in anticipating their behavior, chumming them into a precise location, and carefully choosing the moment for tightening the snare.

In Micronesia, the snare is the most common implement used in capturing sharks (Figure 2). The practice is virtually ubiquitous in the southwestern Pacific, though the designs of snares vary from place to place. Curiously,

Figure 2 Use of the shark snare, with lure (New Ireland), illustrated on a New Guinea postage stamp.

there are no records of the practice in the Marshall Islands (Anell 1955), and it is rare in the Solomon Islands, where sharks are not generally caught, for religious reasons. (They are considered incarnations of dead ancestors; they may also be considered as having tutelary relationships to a village or individual, helping in fishing for bonito, protecting endangered fishermen, and so on (Starzecka and Cranstone 1974).)

The review of snaring methods by Anell (1955) is quite detailed, and the illustration on a recent New Guinea postage stamp (Figure 2) gives a clear idea of the procedure as practiced in New Ireland. Once the shark has been lured by the bait into proper position, the slip-knot noose is drawn tight and the shark tows a propeller or float attached to the noose until it finally becomes exhausted. In some localities, rattles may be built into the snare, in addition to the chemical stimuli employed.

Another instrument for capture or killing sharks, depending on chemical stimuli for its operation, is the shark gorge. It is essentially a strong double-pointed stick, about 0.5 m (20 in.) long. An amputated tentacle from an octopus is attached to the gorge and serves as an odor lure. Since these devices have not been as well documented as the various types of shark snares, a Fijian explanation is included here with translation (Gatty 1978):

E vesu e dua na liga ni kuita e na i sua.
(An arm of octopus is tied to the gorge.)

Ni sa dre na qio nai sua
(when the shark pulls the gorge)

Sa qai cavuka na wa ni kuita
(the string for the octopus breaks)

Ka lutu tani yani
(and falls away)

Ka mani vakavuni me curu
(and in a concealed way the gorge enters)

Ka ciqi vakababa na i sua e nua gusi ni qio
(and slides horizontally in the mouth of the shark)

Ka dredre kina me lutu tani mai
(and only with difficulty would come out).

Gorges are employed, with lines attached, as mechanisms to "hook" and retrieve sharks; in other cases, the gorge is used merely to damage or kill sharks that are judged to pose threats by their territorial or otherwise aggressive behavior. A more extensive survey would probably reveal local variations of technique.

Naturally Occurring Shark Repellents or Toxins—A final consideration, concerning exploitation of the chemical senses, is the possibility of naturally occurring substances affecting olfaction of sharks. Attention to this possibility has been increased by the finding that a fish in the Red Sea

(*Pardachirus marmoratus*) produces a mucus which repels sharks (Clark and Chao 1973). *Pardachirus* and a closely related genus (*Aseraggodes*) occur along the eastern coast of Australia, but no local observations of their shark-repelling capacities were encountered. However, another fish (*Diploprion bifasciatum*) is known to exude abundant and bad-tasting mucus when disturbed, and in several localities of the Northern Territory and Great Barrier Reef it is reported to repel sharks and other potential predators. Several invertebrate animals, especially holothurians (Bakus 1973), are used in Micronesia to poison fish, including sharks, and observations of their avoidance by sharks are common.

To date, no systematic or controlled studies have been carried out of fauna which might be producing "natural repellents" in the South Pacific. Even with the most frequently encountered claims about this matter, no data of a scientific nature appear to exist. For example, in Micronesia and northeastern Australia, one encounters claims that the "ink" squirted during escape and protective behavior by octopi repels predators, including sharks. A tank label in the Honolulu Aquarium goes so far as to claim that octopus ink ". . .deadens the predator's sense of smell." If true, this would have obvious physiological and practical importance, but local scientists were unaware of any adequate studies conducted on the possibility (Tester, personal communication). This should be a fertile area for future investigation in marine pharmacology, toxicology, and behavioral studies.

RECOGNITION OF BEHAVIOR PATTERNS IN SHARKS

Many Pacific Islanders show an awareness of shark behavior patterns, and an ability to discriminate among them, that seems uncanny. Identification of species and activity patterns on the basis of their swimming sounds underwater, mentioned above, is only one example. Visual recognition of shark activity patterns is also acute.

Benchley (1873), in one of the earliest documentations of this matter by a European explorer in the South Pacific, was puzzled by the fact that local swimmers did not always bother to avoid sharks. Between Tonga and Palmerston Island, he encountered an instance when islanders swam ". . . among them (tiger sharks) without manifesting any apprehension." The implications of this appeared to be that the islanders knew when the tiger sharks were in an aggressive "mood," or possibly that the sharks were not ". . . as dangerous as they are supposed to be." (It is difficult to imagine how Brenchley could have drawn the latter conclusion when the frontispiece of the book shows a particularly gory painting, from the Solomon Islands, of shipwrecked men doing battle with, and being eaten by, sharks!) That aggressive behavior can be anticipated or predicted by watching the behavior of potentially dangerous sharks is confirmed by experienced fishermen in many parts of Micronesia. Bending of the back and extension of the pectoral fins of a large shark are considered warning signals of imminent attack, and prompt divers to leave the water, although they might have appeared

indifferent to the same shark moments before. It is noteworthy that these warnings are virtually identical to those discovered in field studies by Johnson and Nelson (1973).

A striking bit of evidence concerning recognition of the aggressive posture of large sharks was found in a wood carving from the Solomon Islands, in the collections of the Museum of the Northern Territory, Darwin, Australia. The carving was a good representation of a shark in agonistic display posture, holding the body of a man in its mouth. The instructive point could hardly be missed. (Unfortunately, this carving was destroyed, along with the museum, in the disasterous Darwin cyclone of December 1974, and since no comparable artifact has yet been located in the collections of other museums, it is impossible to include an illustration.) Dr. Colin Jack-Hinton, curator of the Museum of the Northern Territory identified this carving as an illustration of the "Karimanua" legend, in which the victim is attacked by a shark housing the aggressive spirit of a jealous brother.

As with any myth, the full significance of this can be appreciated only by recognizing that it is a "dramatic shorthand record" of some actual events or possibilities, serving to communicate, teach, or assist the society that perpetuates it (Graves 1959). The superstitious component of the Karimanua myth is obvious. Underlying it, however, the model of a shark, combining agonistic display behavior with a fearful potential result of that behavior, becomes a highly instructive device, communicating an important fact of natural history to as many generations as retain the mythology and associated artwork. Other useful shark models are noted below.

SHARK FISHING ON FAIS—A CASE STUDY

The complex knowledge that underlies human practices relative to sharks in the southwestern Pacific can best be appreciated on the basis of a more thorough analysis of a single locality. The situation on Fais, in the Yap district, western Caroline Islands, is a particularly striking example of a shark-oriented culture.

Fais (also called Feis or Tromelin) is approximately 230 km (145 mi) east of Yap at lat. 9°46′N., long. 140°31′E. It has an area of 2.8 km^2 and a population of about 250. It is an elevated coral island, surrounded by vertical or undercut cliffs 14 to 20 m (45-65 ft) high. The coral reef development around Fais is very scanty, and the typical reef fish thus are scarce. Kramer (1937) reproduced an early sketch chart and provided some brief descriptions of fishing methods used by the islanders. Forsberg and Evans (1969) made a botanical survey of Fais; they also noted the local agricultural and mining activities on the island.

The people of Fais fish specifically for sharks, preferring them to other fishes. The origins and antiquity of this specialization are not known with certainty but may be related to the relative unavailability of reef fish. The argument raised against this interpretation is the situation on Satawal, another island in the Yap district that lacks a surrounding lagoon and has a

scarcity of fish; people from Satawal have consistently met an important part of their protein requirements by sailing to an atoll about 75 km (47 mi) distant and exploiting the abundant fish resources there (McCoy 1974). Whatever the full explanation, the renown of Fais for shark fishing, as well as its cultural preoccupation with sharks, is spread throughout Micronesia. Sharks are not only eaten but are also used as the most valuable gifts, art motifs, etc., on Fais.

Training for Shark Fishing—Carved sharks, such as the one shown in the author's photograph accompanying this article, are commonplace toys of children in Fais, and young boys maneuver the sharks, together with model boats, much as urban children in the United States maneuver model cars and trucks. Although it is difficult to know exactly what knowledge about shark behavior is gained at that stage, the play undoubtedly contributes to the eventual expertise of many young shark fishermen.

When boys are about nine years old, their fathers start taking them out in canoes to watch shark fishing. Several years of observation are considered essential before a boy is allowed to participate outside a canoe. Older men judge the competence of each boy, and at about the age of 12 a boy may be trusted to fish for his own shark in the usual way. Even then, the beginner customarily starts with a small hook that is more likely to catch one of the smaller sharks.

Shark Fishing Techniques—The adult shark fishermen swim out over deep water, hanging onto a log or stout pole about 150 to 200 mm (6-8 in.) in diameter. They carry a line about 61 m (200 ft) long, with a wire leader and steel hook at the end. The hook is baited with any kind of small fish. As soon as the fishing line is thrown out, the man splashes in the water with his hands to attract the sharks. Individual sharks may be spotted underwater and pursued by a fisherman, who thus increases his chances of obtaining a prize specimen.

Once the shark is hooked, the fisherman pulls the line over one end of the log, in order to bring the shark's head up against the end of the log. If the shark is a hammerhead, the lateral projections of the head are bent ("broken"), putting out of operation the visual, olfactory, and other sensory inputs from the head extensions. The fishing line is used to tie the body of the shark to the log. During the tying-up procedures, several other fishermen may help; since this is an everyday type of fishing, involving many people, there are many experienced men if help is needed

Species of Sharks Caught—Three kinds of sharks are reported to be most common among the catches around Fais. From outline sketches and photographs, Fais islanders identify these as (a) "doap"—hammerhead, *Sphyrna*, (b) "poub"—whitetips, *Triaenodon*, and (c) "pougle uch"—a species of gray shark, not completely identifiable from the descriptions. Four- to six-foot sharks are commonly captured, but six-foot sharks are considered about the maximum size that can be taken in this way. Fishermen from Fais report that the sizes of sharks caught seem to be getting

smaller, and some express concern about whether "new sharks" will come to the vicinity of the island as a larger proportion of local sharks are taken. More adequate data on shark behavior will be needed to plan ecologically sound methods of meeting the people's nutritional needs.

Risky as this fishing method may appear, the careful and prolonged training of young fishermen evidently pays off. All informants agreed that they knew of no injuries inflicted by sharks on Fais islanders. By contrast, several volunteered accounts of serious injuries, including losses of hands or feet, sustained by Japanese fishermen who fished for sharks from boats in the area of Fais. "When the shark is known truly good," summarized one informant, "injury is not possible"—a viewpoint that seems to justify hope, along with a lot of patience and further study, for neophytes from other cultures who have reason to work with sharks.

SUMMARY

Among island cultures of the southwestern Pacific, methods which are effective for attracting, repelling, or anticipating the behavior of sharks have been developed through many generations of trial-and-error experience. Procedures for luring sharks by acoustic signals, exploiting chemical baits, and inactivating sensory inputs show detailed insights into the sensory biology and behavior of sharks. In addition, trained abilities to recognize certain shark species and their behavioral patterns, on the basis of underwater sound and visual cues, have been developed in some cultures. The effectiveness and predictive accuracy of some of these methods suggest that careful analysis of local knowledge and practices might suggest useful approaches for further exploration by controlled scientific methods.

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THE EFFECTS OF FASTING CONFINEMENT

ON *SQUALUS ACANTHIAS*

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> The elasmobranchs are interesting in that they appear more susceptible
> to stress than routine laboratory research animals such as rats, mice,
> cats, monkeys, and man. Why sharks and rays, an evolutionarily suc-
> cessful group of animals, should be so susceptible to so many forms of
> stress, many of which are considered to be so slight as to still render
> them experimentally normal, is not answered.
>   Rasmussen and Rasmussen (1967)

Selachians that survive the rigors of capture often fail to adapt to pro-
longed confinement. Representatives of only 6 of the 17 families of sela-
chians have been consistently maintained in captivity for periods longer than
a year (Clark 1963). These may be characterized as generalized carnivores
found in coastal waters; they represent the more primitive of extant families
(White 1937).

The more specialized members of both the galeoid and the squaloid line-
ages fail to adapt to confinement. Those that survive capture cease normal
feeding, at least temporarily (Essapian 1962), and most experimental studies
have been conducted on animals recently subjected to the traumas both of
capture and subsequent starvation. Several authors have stated or implied
that the captive population differs from the wild (Rasmussen and Rasmussen
1967; Dawson 1933; Esapian 1962; Murdaugh and Robin 1967), but no
attempt has been made to determine the significance of the differences.

This study attempts to assess the effects of confinement and starvation on
the spiny dogfish, *Squalus acanthias*. Captive *Squalus* are popular research
animals and have contributed significantly to our understanding of selachian
physiology. The conditions of confinement were chosen to approximate
those at a typical marine research facility.

This report considers the behavioral, histological, and physiological
changes that accompanied confinement. These are integrated to provide
guidelines for evaluating *Squalus* as an experimental animal. Understanding
the changes brought about by confinement and starvation is vital to evalua-
tion of data collected from animals held under these conditions.

MATERIALS AND METHODS

Field research was conducted in the Isle of Shoals, off Portsmouth, New
Hampshire. A substantial population of *Squalus* appears here in mid-June,
when surface temperatures approach 14°C.

A variety of capture methods were employed, including gill netting, long-
lining, and trawling. Animals obtained through trawling were too severely
injured to survive for more than a few hours. Autopsies indicated severe
internal injuries and hemorrhaging.

Animals were held in floating pens, moored in 15–20 feet of water. Median
partitions divided the pens into 10 ft × 5 ft × 6 ft (3.0 m × 1.5 m × 1.8 m)
compartments. During 1971, animals were identified by notches made in
their fins; in 1973, small plastic tags were affixed by metal wires inserted
through the first dorsal fin or the caudal peduncle. Tag shape designated the

experimental group, and tag numbers identified individual animals. Seventy-eight animals were used in this experimental series. All were mature females, 49–91 cm in standard length; several were gravid, bearing pups estimated to be between 3 and 15 months *in utero*. Fewer than 16 animals were held in a pen at any one time. *Squalus* generally refuse to feed in captivity (Sargent, Gatten, and McIntosh 1971, 1972; Murdaugh and Robin 1967). Attempts to induce feeding were unsuccessful, and the sharks fasted until they were sacrificed or died in the experimental enclosure.

In 1973 a record was kept of the water temperature, and water samples were taken at intervals to detect any alterations in salinity.

Four groups were utilized:

I. 1971; 20 animals—Blood samples of 1–3 ml were taken at intervals of 3 to 8 days from time of capture to time of death. At random intervals, animals were removed and sacrificed for tissue samples.

II. 1973; 16 animals—Blood samples were taken from each animal at 5-day intervals up to the time of death.

III. 1971, 1973; 11 animals—Sharks were held undisturbed; individuals were randomly sacrificed for tissue and blood samples.

IV. (1971, 1973; Controls)

a. Wild population: Animals were caught and immediately sacrificed (within 5 min) for blood and tissue samples (19 animals).

b. Experimental population: Animals were placed in the pen and left undisturbed until they expired naturally (12 animals).

Blood samples were drawn from the caudal vein using sterile, disposable syringes with 18- or 20-gauge needles. The volume of blood per sample was approximately 3.5 ml. Hematocrit determinations and colorimetric assays for serum urea, cholesterol, protein, glucose, and total lipid were performed on fresh sera. Aliquots of sera (1–1.5 ml) were frozen for transport to Cornell University; after thawing, these were assayed for serum sodium, potassium, chloride, total osmolarity, urea, iron, and calcium.

Total serum osmolarity was determined on an Advance Instrument Wide-range Osmometer. Serum sodium and potassium were analyzed by flame photometry, and serum calcium and iron levels were assayed by atomic absorption spectrophotometry. Serum chloride levels were determined using an IL-279 chloride analyzer. Glucose and urea were assayed following the procedure of Hanok (1969) with the aliquot for urea determination diluted 1:10. Serum cholesterol was analyzed by a modification of the Lieberman-Burkhardt technique (Huang et al. 1961). Total serum lipids were assayed by the turbidimetric method (Davidsohn and Wells, 1965) and the units converted to mg% lipid by the conversion factor used in mammalian serological assays. Total serum protein was determined by the biuret method.

For blood smears, 0.5 ml of blood were drawn from the caudal vein into a heparinized syringe. Smears were made upon arrival in the laboratory, air-dried, and stored. These were subsequently stained with Wright's stain. When tissue samples were desired, following the removal of a blood sample the

shark was removed from the water and sacrificed by transsection of the spinal cord and destruction of the brain.

Animals were randomly selected for sacrifice. The number of animals sampled and the periods of time in the experimental pens are given in Table 1. Samples of kidney, rectal gland, esophagus, stomach, spiral intestine, spleen, gonad, liver, pancreas, and abdominal wall were removed following the sacrifice of an animal. Small pieces (2.5 mm^3) of tissue were fixed in either 10% neutral buffered formalin (ph = 6.9 in either seawater or elasmo-branch ringers, approximating the osmolarity of the plasma),* Baker's for-malin (pH = 7, isosmotic), aqueous Bouin's solution (in elasmobranch ring-ers), or Helly's fluid. Sectioning was performed after embedding in paraffin or glycolmethacrylate.

Frozen sections were made after being embedded in water, Tissue Tek, or 10% gelatin. The sections were stained with Sudan Black B, Sudan IV, or Nile Blue A (Humason 1967).

A qualitative assay for cholesterol in the interrenal gland was designed, using the Microphotoautomat complex of a Wild M-20 microscope (Wild Heerbrugg, Ltd., Ch-9433 Heerbrugg, Switzerland). This apparatus monitors light intensity at the source and at the attached camera; the results are

Table 1. Number of animals sampled and days in captivity.

Days in captivity	Number of animals
0	13
1–4	3
5–8	3
9–12	5
13–16	8
17–20	4
21+	1

Total		
Exp.	24	
Control	13	

*	mm/l
NaCl	310
Urea	430
KCl	4
NaHCO$_3$	4.5
MgCl$_2$ · 6H$_2$O	2.5
NaH$_2$PO$_4$ · H$_2$O	.5
CaCT$_2$ · 2H$_2$O	2.5

indicated on calibrated gauges. Frozen sections of interrenal tissue (20 μm) were floated on 3% hydrogen peroxide, as in the Schultz technic (Thomas 1966). After 3 min, a section was transferred to an acid-cleaned slide. Three drops of Liebermann-Burchard reagent (Huang et al. 1961) were placed over the section, which was covered with a clean coverslip. A red filter was placed below the condenser of the microscope, and the light intensity was adjusted to a standard level at the source. The microscope was focused on the center of the gland using the 20X objective (magnification = 200X); a "blank" value was obtained by recording the light intensity reaching the camera. Color development was completed in 30 min, and transmitted light was recorded again at that time. A "Color Index" was then derived by subtracting the blank value.

Assuming a consistent effect of confinement for all animals, differences in individual history would affect the animal's survival. Animals that have not fed in the wild enter captivity already influenced by starvation, while well-nourished animals begin their fasts only when placed in the experimental enclosure. Patterns of change in monitored parameters are masked by this asynchrony if plotted against "days in captivity"; this problem was eliminated by plotting parameters against "days before death."

In addition to nutritional differences, interindividual variation in "normal" physiological and metabolic parameters in fishes has been noted repeatedly (Srivastava 1970; Cordier, Barnoud, and Brandon 1957; Idler, O'Halloran, and Horne 1969; Burger 1967b; Denis 1922; Scott 1921; Hartman, Lewis, Brownell, Sheldon, and Walther 1941; Marsh and Gorham 1906). These effects were minimized by evaluating patterns through covariance analysis of the data. This method computed trends based on the patterns of change for individual animals. These analyses were performed by Mr. William Bell of the Cornell University Biometrics Department and relied on programs of the Statistical Analysis System (A. J. Barr and J. H. Goodnight, North Carolina State University).

RESULTS

Behavioral Observations

Behavioral changes were both marked and stereotyped over the time in captivity. Introduction into the pen was followed by a variable period (minutes to hours) of inactivity. Mortalities were frequent over this period, presumably from the stresses of capture and transport, since none of the animals had obvious injuries. This period of inactivity preceded several days of almost continual activity. The sharks swam strongly with infrequent pauses on the floor of the pen; these "rests" were observed principally during daylight hours. Blood samples taken at this time clotted rapidly. Although no fighting was seen, scarring and torn fins were common at this time; aggressive interactions probably occurred at night, when swimming activity appeared to be more vigorous, but detailed observation was more difficult.

With the passage of time, periods of inactivity increased both in frequency and duration. During isolation for sampling, the animals struggled weakly or not all all. Eventually, the sharks were unable to right themselves, and lay ventral side up. Abortions were common at this time. Subsequently, the animals became rigid and appeared unable to move any but the pharyngeal muscles necessary to maintain a weak respiratory current across the gills. Clotting of blood samples was extremely slow, and the serum frequently showed a distinct discoloration. Death followed within 24 h. Dissection indicated extreme emaciation, with wrinkling of the skin.

Histological Alterations

Figure 1a, 1b illustrates the effects of prolonged starvation on muscle fibers of the ventro-lateral abdominal wall. The muscles have atrophied, intercellular fluid has increased, and cellular organization appears disrupted.

No consistent alterations were observed in the mucosa of the esophagus, stomach, or spiral intestine. Sections of liver stained for glycogen showed a faintly positive reaction in one of the sections from a wild control; all other tests were negative.

The exocrine portion of the pancreas was greatly modified. Figure 1c, d compares the acinar cells of a freshly caught animal with those of a shark sacrificed after two weeks in captivity. A marked reduction in the size of the acinar cells occurred, accompanied by cytoplasmic vacuolation. Cell height was reduced, partly due to decreases in secretory granulations and nuclear size.

No histological changes were seen in the opisthonephroi of experimental animals until they were near death. Figure 1e, f compares the appearance of the kidney of a control animal (group IVa) with that of an animal sacrificed after 18 days in captivity. A dense precipitate was frequently present in the tubules. Tubular damage was evident, and disaggregate cells and cellular debris occasionally occluded a tubule.

Measurements were taken of the nuclear diameters of tubular cells in the rectal glands of eight animals; the results are presented in Table 2.

A significant decrease in nuclear diameter occurred during the experimental period. This was accompanied by increasing density and basophila of the chromatin in the nucleus, an increase in the density of the cytoplasm, and some cytoplasmic vacuolation (Figure 1g, h). Similar observations generally accompany a decrease in secretory activity (Ham 1969).

The interrenal organ of Squalus acanthias is an elongate structure located dorso-medial to the opisthonephros. The cells of the gland are arrayed in anastomosing cords, which are delineated by narrow, tortuous vessels. In histological preparations, after paraffin embedding the cells of the normal gland appear columnar in form, with basal nuclei which contain prominent nucleoli. The lightly basophilic cytoplasm appears lacy and vacuolated. It can be demonstrated, using frozen sections of formalin-fixed tissue, that these cells contain abundant lipid inclusions. Histochemical procedures indicate the presence of cholesterol.
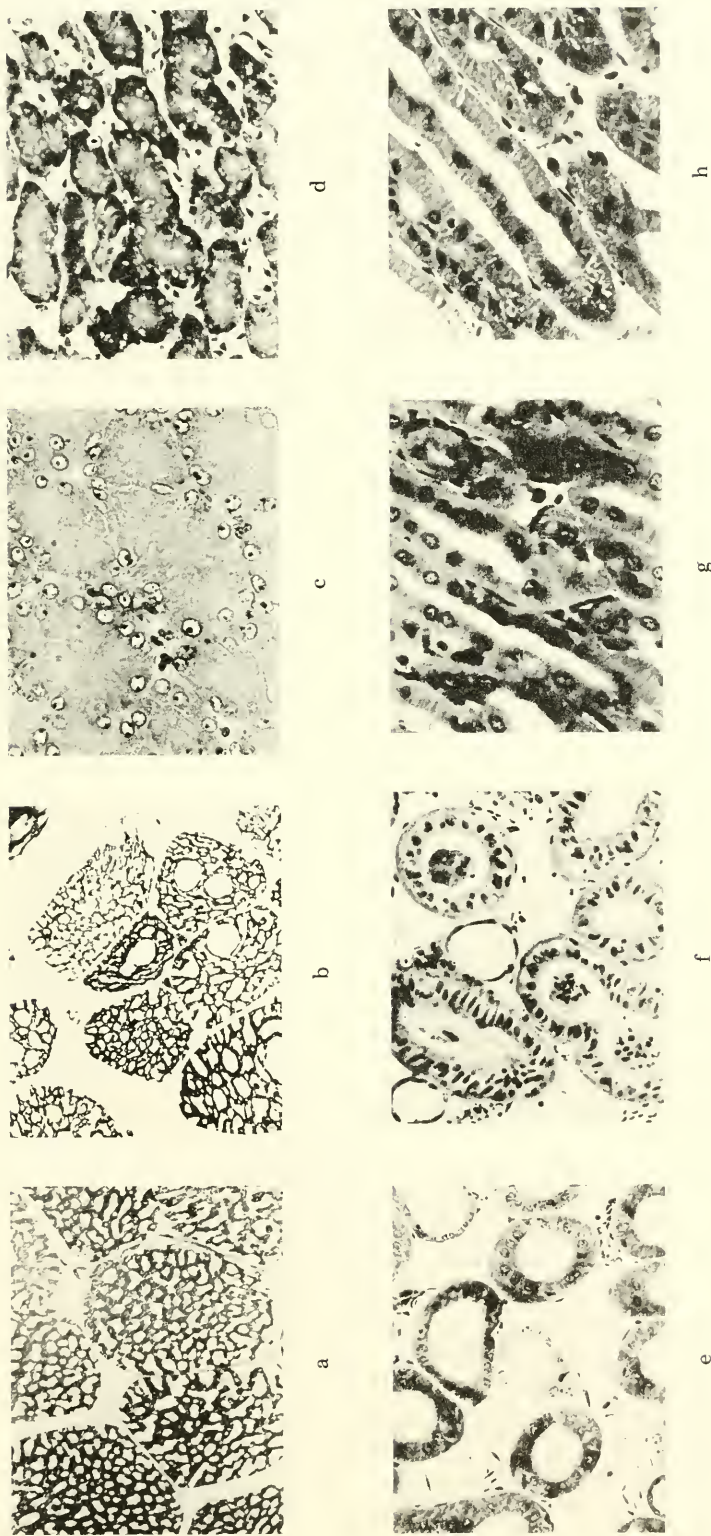
Figure 1 (a) Abdominal wall of a freshly caught animal (formalin fixed, glycol-methacrylate embedding, 4 µm); hematoxylin and phloxine, ×200. (b) Abdominal wall of an animal that died in the pen after 18 days in captivity (processed as in 1a); hematoxylin and phloxine, ×200. (c) Pancreas of a normal animal, showing acinar cells of exocrine pancreas (Bouin's fixed, glycol-methacrylate embedding, 4 µm); hematoxylin and eosin, ×250. (d) Exocrine cells of the pancreas of an animal sacrificed after 13 days in captivity (processed as in 1c); hematoxylin and eosin, ×250. (e) Kidney of normal animal (processed as in 1a); hematoxylin and eosin, ×200. (f) Kidney of animal sacrificed after 4 days in captivity (processed as in 1a); hematoxylin and eosin, ×200. (g) Rectal gland of normal animal (processed as in 1a); hematoxylin and eosin, ×200. (h) Rectal gland of animal sacrificed after 18 days in captivity (processed as in 1a); hematoxylin and eosin, ×200.

Table 2. Alterations in diameter of nuclei in cells of the rectal gland.

| | Category of Shark | | |
	Freshly caught controls	Sacrificed while active	Died in pen
Diameter			
Average nuclear diameter (μm) N = 400	12.2 11.6	10.1 10.4 10.3	10.1 9.9 9.3

Results of Tukey test for significant difference at 0.05 level. (Those underlined are not significantly different.)

Figures 2 and 3 present the alterations in the histological character of the gland of experimental animals. The vascularity of the glands of animals sacrificed while still active was invariably greater than in control animals, and the nuclei of the parenchymal cells were enlarged (Table 3). As the condition of the animal worsened, the height of the glandular cells decreased, and they became cuboidal in form. These cells appeared to posses a homogeneous cytoplasm, and contained little demonstrable lipid. Nuclei became smaller and more densely stained, and shifted towards a central or apical position. The cholesterol content of the gland decreased markedly (Table 4). The interrenals of animals sampled immediately after death in the pen or sacrificed when death appeared imminent (indicated by immobility and low hematocrit values) were greatly altered. The lipid content of the gland was greatly reduced, and the cells showed little cytoplasmic vacuolation. Numerous blood cellular elements were present, and polymorphs no longer appeared restricted to vascular spaces. In the most extreme instances, the gland was totally disorganized. Thus, the histological picture indicates activation of the gland followed by gradual exhaustion of the parenchymal cells (Cain 1950; Long 1947; Moon 1961; Weatherley 1963).

Changes in hematocrit values were marked over the experimental period; they fell steadily from an average of 20 to percentages as low as 2–4 (see Figure 4). A change in the character of the hematopoeitic organs occurred as the hematocrit declined. The red pulp of the spleen contained progressively fewer mature red blood cells; erythroblast and hemocytoblast mitoses became more frequent in all hematopoeitic organs. Erythroblasts, and ultimately hemocytoblasts, made up a significant portion of the cells of the circulating blood (up to 24%, not including immature erythrocytes). Red blood cells with aberrant shapes (i.e., of exceptional size, spherical or scalloped in profile) were apparent in blood vitally stained with brilliant cresyl blue or new methylene blue. The polymorphs often contained a dense, brownish pigment; a pigment of similar appearance was also found in the
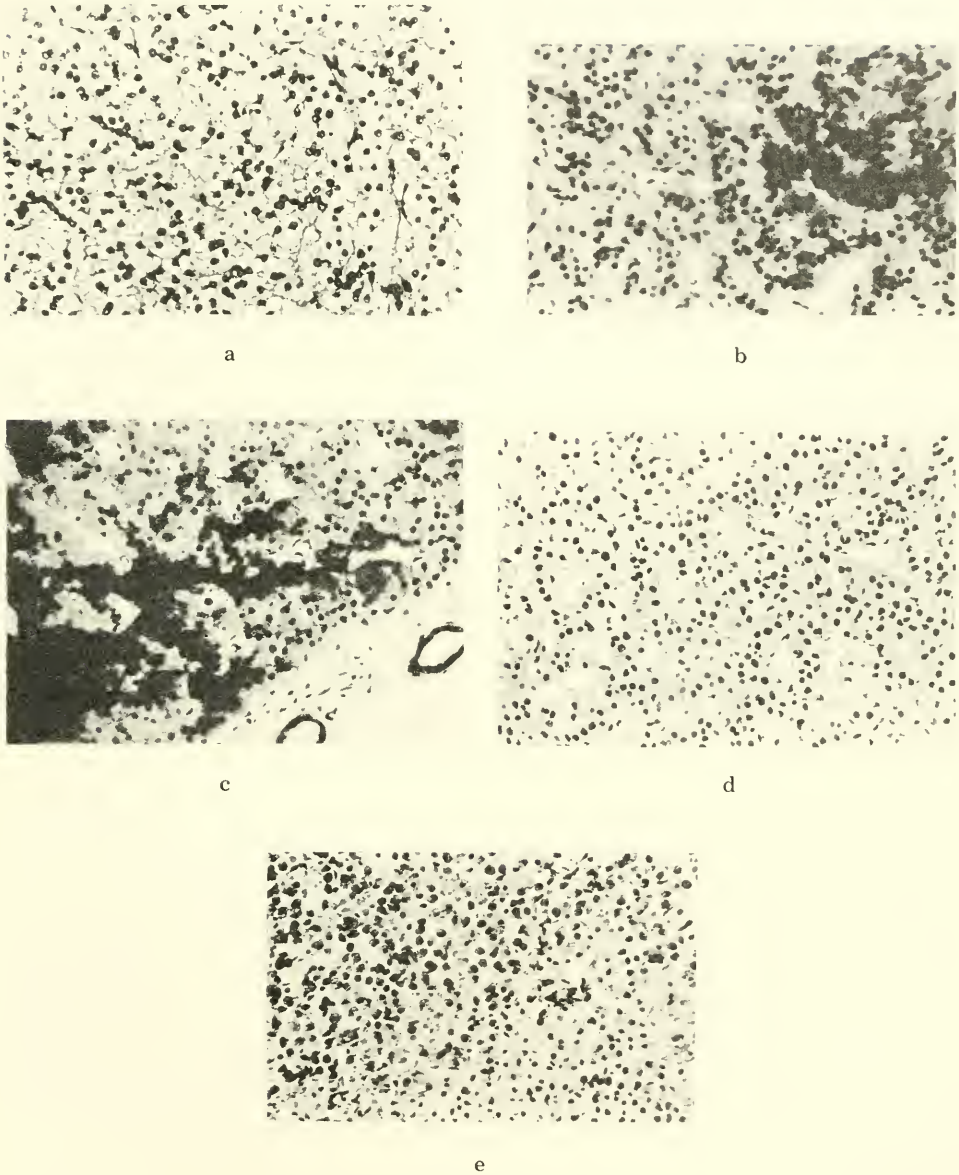
Figure 2 Alterations in the histological appearance of the interrenal glands of captive *Squalus acanthias*. (a) Parenchyma of the normal gland (formalin fixed, glycol-methacrylate embedding, 4 μm); hematoxylin and eosin, × 125. (b) Interrenal from a shark sacrificed after 14 days in captivity (processed as in 2a); hematoxylin and eosin, × 125. (c) Interrenal from an animal sacrificed after 18 days in captivity (processed as in 2a); hematoxylin and eosin × 125. (d) Interrenal from an animal sacrificed when immobile, after 9 days of captivity (processed as in 2a); hematoxylin and eosin, × 125. (e) Interrenal from a shark that died in the pen after 18 days in captivity (formalin fixed, para-plast embedding, 4 μm) × 125.
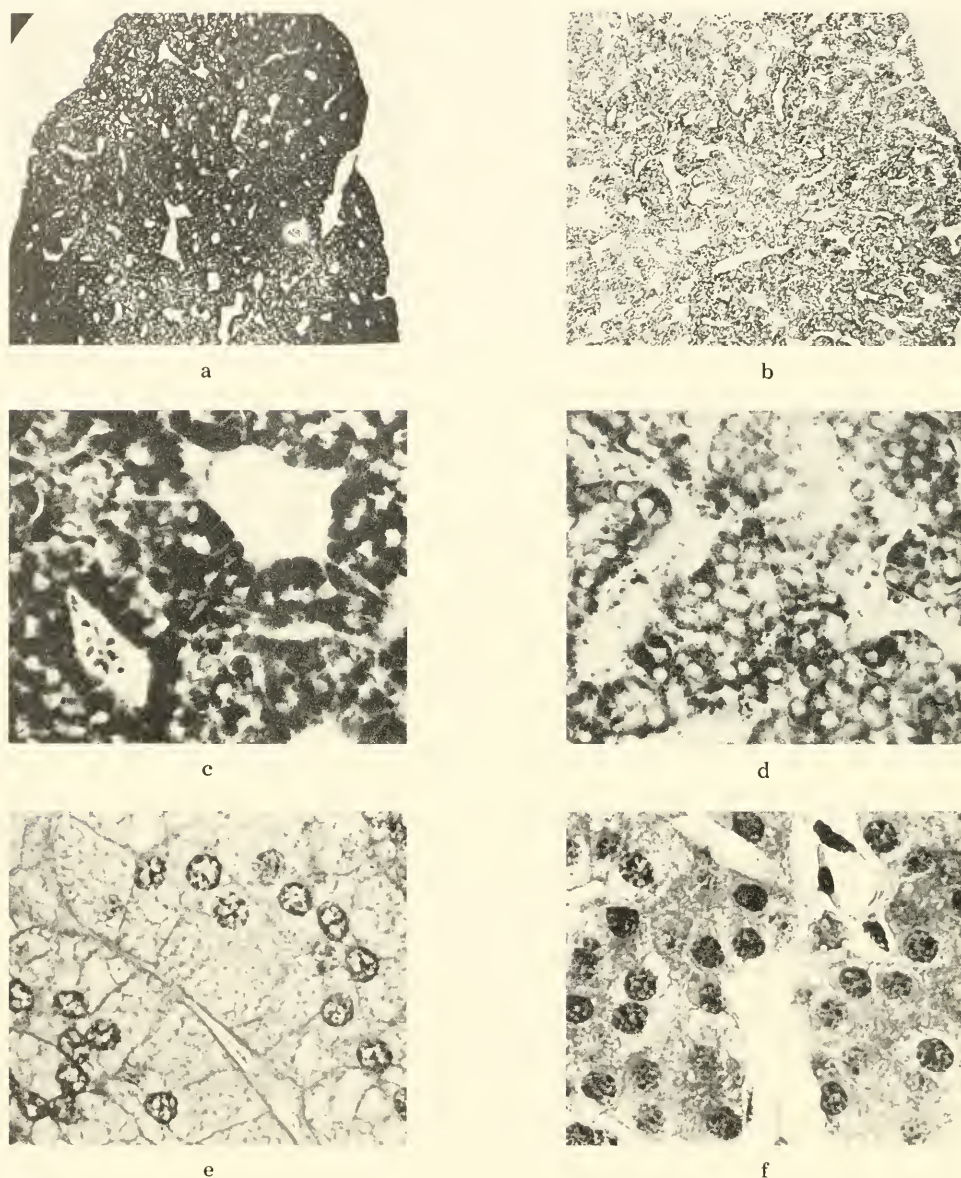
Figure 3   Alterations in the histological appearance of the interrenal glands of captive *Squalus acanthias*. (a) Interrenal of a freshly caught animal, stained for lipid contents (formalin fixed, frozen section, 10 μm); Sudan Black B, $\times$40. (b) Interrenal of an animal sacrificed after 18 days in captivity, stained for lipid contents (processed as in 2a); Sudan Black B, $\times$40. (c) Interrenal of a freshly caught animal, stained for lipid contents (processed as in 3a); Sudan Black B. $\times$250. (d) Interrenal of an animal sacrificed after 18 days in captivity (processed as in 3a); Sudan Black B, $\times$250. (e) Parenchymal cells of a freshly caught animal (formalin fixed, glycol-methacrylate embedding, 4 μm); hematoxylin and eosin, $\times$500. (f) Parenchymal cells of an animal sacrificed after 9 days in captivity, showing cells with low lipid content and homogeneous cytoplasm (processed as in 3a); hematoxylin and eosin, $\times$500.

Table 3. Alterations in nuclear diameter in cells of the interrenal gland.

Animal	Mean diameter of nuclei (n = 50)	Result of Tukey test at 0.01 level*
Wild control	8.6μ ± 0.7†	
Sacrificed active	9.7 ± 0.7	Significant
Sacrificed immobile	8.5 ± 1.2	Significant
Died in pen	7.8 ± 0.5	Not significant

*As compared with the preceding mean diameter.
†Mean ± standard deviation.

Table 4. Gland cholesterol, modified Schultz reaction.

Animals	Color index
Wild population controls	19
	17
Sacrificed after 6–14 days in captivity—actively swimming	20
	19
	19
	21
Sacrificed when immobile	11
	10
Samples collected after death in pen	3
	0

reticuloendothelial cells of the sheathed arteries. This pigment did not stain for iron with any of the tests used (Gomori's, Hutchinson's, Turnbull Blue, or bathophenanthroline techniques, following buffered formalin fixation). Hemmeter (1926) described a pigment of similar appearance in the arterial sheaths in the spleen of *Alopias*, and attributed it to products of erythrocyte destruction. This pigment has the histochemical characteristics of hematoidin. Figure 5a–f summarizes the alterations observed in the spleens of experimental animals.

Little agreement exists in the literature concerning the origins and identities of other hematological elements of elasmobranchs. The following cell types, which will be described in detail elsewhere (Martini, in preparation) will be referred to in this paper: lymphocytes, monocytes, thrombocytes, polymorphs, eosinophils I (fine, rod-shaped granules found in the circulatory

Figure 4 Alterations in hematocrit values of captive *Squalus acanthias*.

channels), and eosinophils II (coarse granules rarely found in circulating blood).

During the experimental periods, lymphocytes became infrequent in the circulating blood, and lymphocytic concentrations in the spleen decreased in size. Large numbers of mature eosinophils II, polymorphs, and (with increasing frequency) eosinophils I appeared in all hematopoeitic centers. Thrombocytes in the blood and thrombocytic mitoses in the spleen increased at first and then decreased in number. Reticuloendothelial cells of the sheathed arteries of the spleen appeared greatly enlarged; mitoses were occasionally observed. These cells often contained dark pigment granules or large, pale-staining inclusions (Zenker's or Bouin's fixed tissue).

Physiological Observations

Table 5 presents the results of assays of sera from freshly caught animals during 1971 and 1973. Data quoted here are within the range of values reported for *Squalus* body fluids by other authors (Maren 1967; Burger 1967; Murdaugh and Robin 1967). Differences significant at the 0.01 level (Student T-tests) exist in serum levels of glucose, cholesterol, and proteins at the time of capture between the wild populations of the two experimental seasons. The summer of 1971 was relatively cool and cloudy; air temperatures averaged 15.8°C during June and 20.2°C in July. Potential food sources included large numbers of mackerel, cod, hake, herring, and euphausiid crustaceans. During the experimental period of 1973, air temperatures averaged 18.7°C in June and 21.3°C in July (U.S. Department of Commerce, Environmental Data Service), and food fish were scarce in the area. Thus the relatively depressed blood glucose and cholesterol levels of animals in 1973 may be related to increased temperature and decreased food supply. Cholesterol generally declines during starvation, and low serum levels

Figure 5 (a) Normal spleen, from a freshly caught animal (formalin fixed, paraffin embedding, 6 μm); hematoxylin and eosin, ×40. (b) Spleen from an animal sacrificed after 13 days in captivity (processed as in 5a); hematoxylin and eosin, ×40. (c) Spleen from an animal that died in the pen after 18 days in captivity (processed as in 5a); hematoxylin and eosin, ×40. (d) Spleen from an animal that died in the pen after 22 days in captivity (processed as in 5a); hematoxylin and eosin, ×40. (e) Reticulo-endothelial cells of the sheathed arteries, normal spleen (formalin fixed, glycol-methacrylate embedding, 4 μm); hematoxylin and eosin, ×500. (f) Cells of the sheathed arteries from an animal that died in the pen after 22 days of captivity (processed as in 5a); hematoxylin and eosin, ×500.

Table 5. Comparison of physiological data from wild populations in 1971 and 1973.

Year	Hct	Chol	Glu	Urea	Serum protein	Total lipids	Na$^+$	K$^+$	Cl$^-$	Ca^{++}	Fe^{++}	T. Osmol.
1971	20 ± 2* 6	117 ± 8 13	98 ± 7 11	379 ± 35 10	1.432 ± .07 13	—	—	—	—	—	—	—
1973	23 ± 2 16	76 ± 6 17	75 ± 5 15	370 ± 11 14	1.928 ± .08 14	809 ± 61 16	242 ± 6 18	2.6 ± .3 17	251 ± 7 15	3.6 ± 1 9	14.7 ± 5.6 7	1073 ± 27 11
	NS†	S	S	NS	S	—	—	—	—	—	—	—
Sea-water 1973	—	—	—				356	8.8	417	10‡	—	930

*Mean ± Sem
†Results of 2-sample T-test for difference between the two years significant at 0.01 level. NS = not significant, S = significant.
‡From Burger (1967).
§Hct = Hematocrit %; Chol = Cholesterol, mg%; Glu = Glucose, mg%; Urea = mm/l; Protein = gm%; Lipids = mg%; Na$^+$, K$^+$, Cl$^-$, Ca^{++} = mm/l; Fe^{++} = M/L; T. Osmol. = Total Osmolarity, mOsmls.

of glucose in wild populations of *Squalus* have been interpreted as reflecting inanition (Patent 1970).

Table 6 presents the data on serum protein levels sorted by method of capture as well as by year. Animals caught at the surface were brought aboard within 5 min of their acceptance of the bait and had little time to struggle. Sharks caught on the bottom remained on the line for up to 12 h. Even with minor hook wounds, the blood loss over this period could be significant. Gill-netted animals, however, seldom sustain any blood loss. Sudak (1960) reported a linear relationship between blood loss and arterial pressure; this would result in a fluid shift from the intercellular space into the plasma, lowering the plasma protein concentration.

Table 7 compares the survival times of experimental (groups I and II) and control (group IVb) populations. Significant differences do not exist for a given year, but survival times do differ over the two experimental seasons.

Table 6. Effects of method of capture on serum protein concentration.

Year	Capture method	Serum protein (gm%)	Results of T-Test*
1973	Gill net (a)	1.936 ± 0.11 6	c, d
1973	Longline (b) (surface)	1.984 ± 0.18 6	d
1973	Longline (c) (deep)	1.542 ± 0.2 3	a
1971	Longline (d) (deep)	1.433 ± 0.07 13	a, b

*Indicated group differs significantly at or below the 0.1 level.

Table 7. Comparison of survival times for experimental and control groups of 1971 and 1973.

Year	Experimental (Groups I & II)	Terminal control (Group III)	Results of 2-sample T-test (0.05)
1971	14 days (16–21) 13	19 days (16–22) 4	NS*
1973	12.6 (8–17) 11	10.7 (9–14) 7	NS

Note: Mean survival time, 1971: 15.4 days
Mean survival time, 1973: 11.2 days
Result of T-test: Significant at 0.01 level
*Not significant.

This is attributed to seasonal differences in temperature and the metabolic reserves of the animals.

Identification of a general trend in total serum osmolarity was difficult due to the variations in chloride and urea levels, reported below, which contribute significantly to the total osmolarity of the serum. Figure 6a presents a scatter diagram of the data, with data points from five animals connected.

Serum levels of sodium and potassium consistently increased over the experimental period. Figure 6b, c presents scatter diagrams of the data points; these figures also present the computer-determined patterns that best fit data collected from all the individuals sampled. Serum levels of both calcium and iron also increased as the animals approached death (Figure 7a, 7b).

Serum chloride levels rose in most of the animals (Figure 7c).

Serum glucose gradually decreased over the period of confinement. Exceptions existed principally among animals that had remained on the longline for prolonged periods. This was presumably due to the attendant exertions; in these instances blood glucose was low at capture but increased by the second sampling. Thereafter it declined. Occasionally, a marked elevation in blood glucose appeared immediately prior to the death of an animal (Figure 8a).

Serum cholesterol levels were variable within the population and, to a lesser extent, within a single animal over time. The general trend was a decline in serum cholesterol as the animals approached death (Figure 8b).

The total quantity of serum lipids fell rapidly, much more dramatically than the serum cholesterol values (Figure 8c).

No statistically significant trend was apparent in serum levels of urea (Figure 9a).

Serum protein concentrations declined steadily during the period of confinement (Figure 9b).

DISCUSSION

Histological Alterations

The only consistent change in the digestive system over the experimental period was destruction of the acinar cells of the exocrine pancreas.

Kern (1966) treated starving *Scyliorhinus canicula* with alloxan and found no lesions in the endocrine pancreas. He did observe degeneration in the exocrine pancreas of all experimental animals. These alterations, and those of the present work, may represent responses to starvation. In mammals, ligation of the pancreatic ducts produces destruction of exocrine cells without damage to the endocrine pancreas (Banting and Best 1922).

Epithelial compression was reported by Oguri (1964) as accompanying regressive alterations in rectal glands of freshwater elasmobranchs, and Chan and Phillips (1967) described cellular vacuolation in rectal glands of low secretory activity.
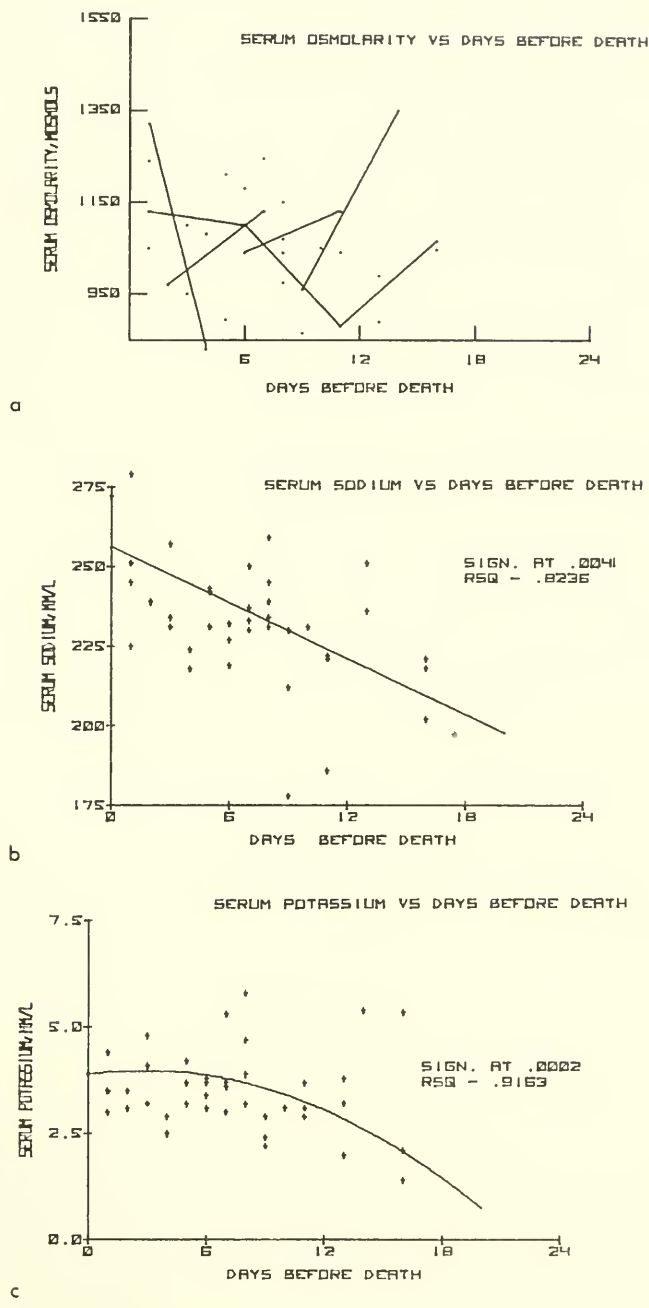
Figure 6 Alterations in serum osmolarity, sodium, and potassium in captive *Squalus acanthias*.
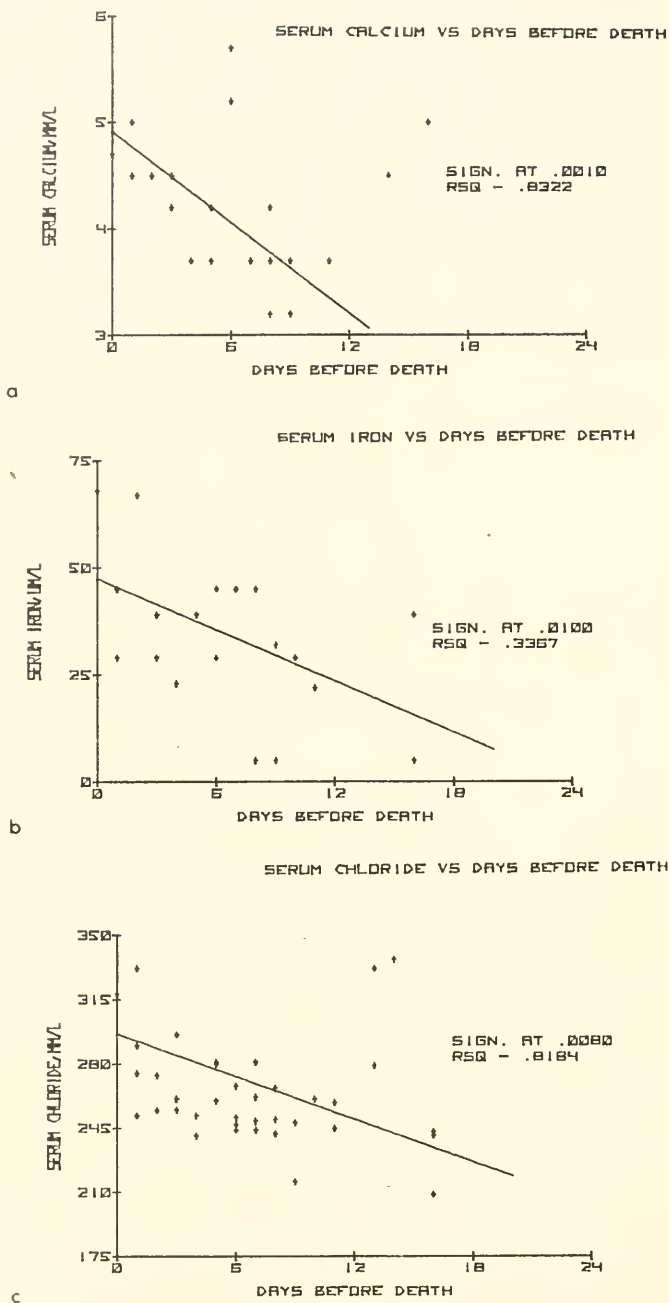
Figure 7   Alterations in serum calcium, iron, and chloride
in captive *Squalus acanthias.*
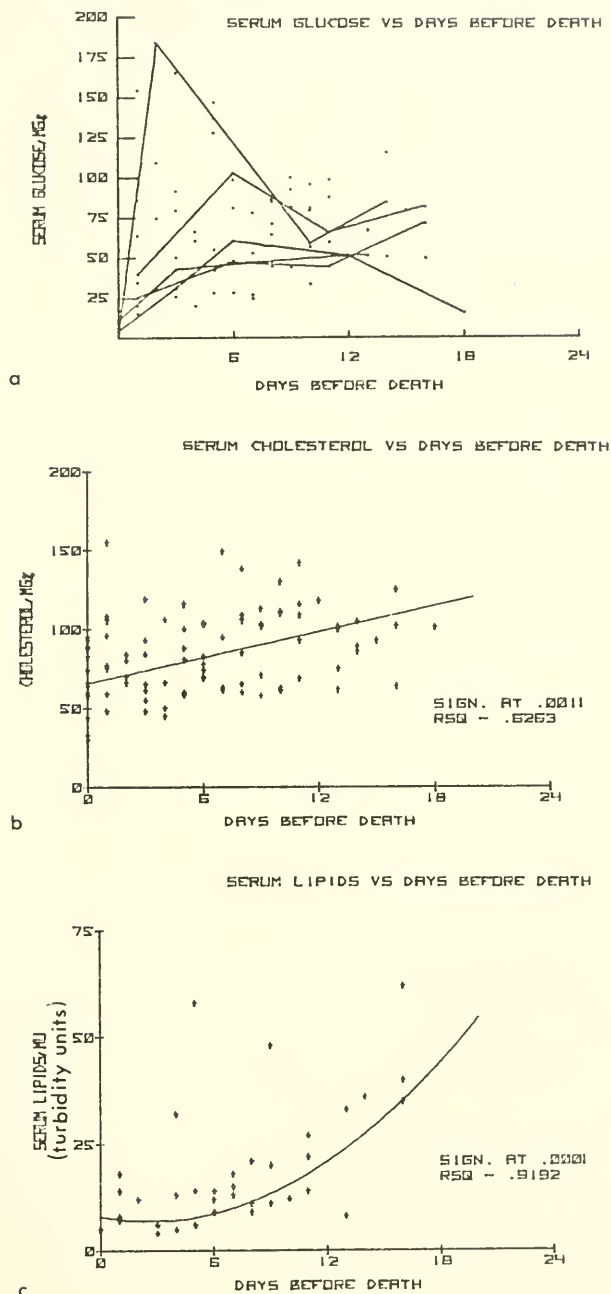
Figure 8 Alterations in serum glucose, cholesterol, and total lipid fraction in captive *Squalus acanthias*.
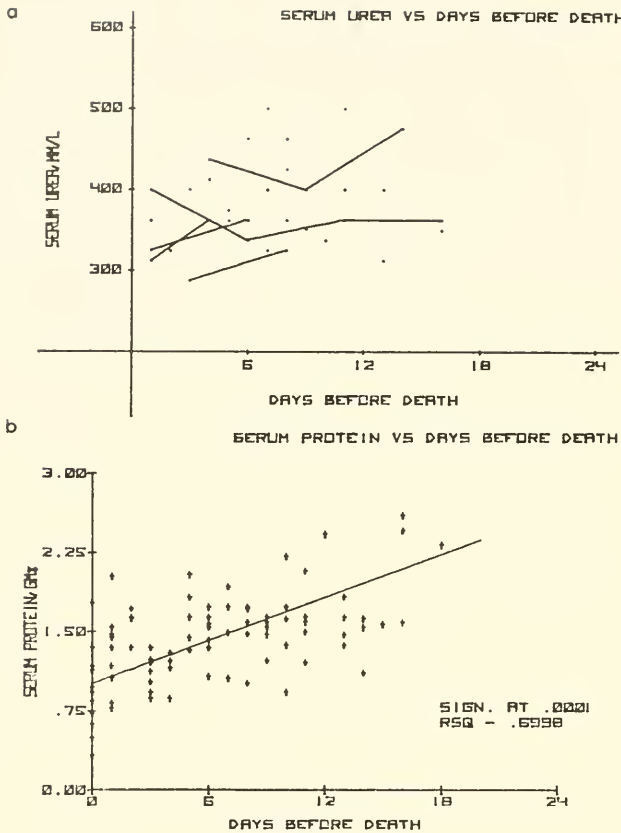
Figure 9   Alterations in serum urea and protein in captive *Squalus acanthias.*

Histological alterations in interrenal tissue accompanying an increase in secretory activity have been described for a number of osteichthyean fishes (Weatherley 1963; Leloup-Hatey 1964; Rasquin and Rosenbloom 1954; Olivereau 1962; Fontaine and Olivereau 1957; Fagerlund, McBride, and Donaldson 1968; Hill and Fromm 1968; Wedemeyer 1969). The sequence described above—(a) increase in vascularity and nuclear hypertrophy, (b) decrease in cell height, lipid content, and cholesterol, (c) increase in basophilic cells with low lipid contents, and (d) disorganization of the gland—is consistent with their descriptions. The basic observations of Dittus (1941) on stimulated interrenals of *Torpedo* are paralleled in this report. However, mitoses of parenchymal cells were not found in *Squalus* interrenals.

Fraser (1929) described lipid secretion in the interrenal of *Scyllium* as a complex process involving a cycle of (a) lobular hypertrophy, (b) rupture, (c) acinus formation, (d) acinus disintegration, and (e) reformation of lobule. Aboim (1946) saw no comparable stages in his specimens but did report hypertrophy of cells followed by rupture and regeneration. No similarity exists with the secretory cycle of *Squalus* interrenals reported here, and further observations should be made on interrenal secretion in *Scyllium.*

In some elasmobranchs, alterations in interrenal morphology exist in accordance with the sexual cycle (Fancello 1937: *Scyllium*; Oguri 1960c: *Narke*; Dittus 1941: *Torpedo*). No correlation between interrenal appearance and reproductive status was observed for *Triakis scyllia* (Oguri 1960c); in the present study the presence or absence of pups, or the relative ages of the pups, did not correlate with the appearance of the interrenals.

Observations reported above indicate lymphocytopenia, thrombocytopenia, and leukocytosis in the blood, with regression of lymphatic tissues and an increase in tissue eosinophils and hypertrophy of phagocytic elements of the reticulo-endothelial system. Degenerative change in lymphatic tissue is a common mammalian response to prolonged corticoid release (Cope 1972). Alterations in the blood cell population of fishes following ACTH or cortisol injection include decreases in lymphocytes and thrombocytes (Weinreb 1968: *Salmo gairdneri*; Weatherley 1963: *Perca fluviatilis*) and increases in neutrophils (Weinreb 1968: *Salmo gairdneri*). In *Lepomis macrochirus*, administration of corticoids caused a hypertrophy of phagocytic cells of the reticulo-endothelial system and an increase in the presence of pigment granules (Fleming and Pasley 1965).

In mammals, eosinopenia in the circulating blood follows corticoid administration (Cope 1972) and is accompanied by an increased sequestration of eosinophils in the spleen (Williams 1968). The present study reports an increase in the eosinophils of both the blood and the tissues. With this exception, the changes in histological appearance of the hematopoeitic tissues resemble those attributed to the anti-inflammatory activity of corticoids found in higher vertebrates.

Ionic Alterations

Little work has been done in examining directly the effects of starvation on elasmobranchs. Hartman, Lewis, Brownell, Shelden, and Walther (1941) reported elevated levels of serum sodium, chloride, and potassium in starved *Raja erinacea*. Similar patterns were reported in later studies on the effects of interrenalectomy on rajids, for both interrenalectomized and sham-operated controls. In addition, this study reported an increase in serum calcium. However, while Idler and Szeplaki (1968) reported increased serum sodium and calcium, they observed decreases in serum potassium and chloride following interrenalectomy.

In a later work, Idler (1969) described pools of corticosteriods in pericardial and perivisceral fluids and suggested that the effects of interrenalectomy had been masked by a slow reentry of these cortical hormones into the circulation. Thus it would appear that earlier studies had in fact assessed only the effects of inanition. The present study agrees with Hartman et al. (1941, 1944) with respect to increases in serum, sodium, potassium, chloride, and calcium.

In mammals, an increase in total body water accompanies sodium retention during starvation or some other stress, and Baldridge (1972) reported an increase in total body water in starved carcharhinid sharks. In those elasmobranch species studied, integumentary permeability to water

increases following ACTH administration (Payan and Maetz 1971). Increased sodium chloride and/or water retention could also be due to decreased secretion of the rectal gland, functional impairment of the kidneys, or decreased sodium excretion at the gills. The interrenal organ is active over the period of starvation (see above); its secretions are powerful mineral-ocorticoids (Idler, Freeman, and Truscott 1967), and the rectal gland has been shown to be responsive to corticoid administration (Chan and Phillips 1967).

The rise in serum potassium is attributed to two sources: (a) an increased catabolism of tissue proteins with concommitant leakage of intracellular potassium and (b) an increase in intravascular hemolysis. A tendency to hemolyze was observed in the studies of Hartman et al. (1941, 1944) but not in those of Idler and Szeplaki (1968). In the present study, rising chloride levels were often accompanied by rising concentrations of serum iron, and the highest potassium values were observed in conjunction with elevated chloride and iron levels. All the observed increases in serum potassium, chloride, and iron are not attributed to hemolysis, however, for on several occasions each increased independently.

The alterations in ionic components observed during the period of starvation thus include increases in serum sodium, calcium, iron, potassium, and chloride.

Metabolic Alterations

The levels of total serum lipids reported here for 16 freshly caught animals, averaging 809 mg%, agree with figures for *Squalus* reported by Lauter, Brown, and Trams (1968) of 792 mg%. Sargent et al. (1971) found much lower lipid levels (125 mg%) in the blood of fasting male *Squalus*; valid comparison with the present study is difficult because the animals were both smaller (1.5 kg vs 3–5 kg) and of the opposite sex. Variation in the lipid content of the serum may exist in the population as a function of the reproductive state of the individuals. The highest lipid levels recorded were found in three freshly caught animals possessing flaccid uteri, which had presumably pupped immediately before capture. These animals had very large ovarian ova. A marked decrease in serum lipid was observed in all animals over the experimental period; much of the decline may represent a decrease in mobilization and transport of lipid components required for the maturation of oocytes. A decrease in gonadotrophin levels is common during starvation in mammals (Dill, Adolph, and Wilber 1964), and atrophy of gonadal tissues follows prolonged adrenocortical activity (Moon 1961). Davydova (1972) has described a cessation of oocyte development during starvation in captive sturgeon, and Rasquin and Atz (1952) reported regressive changes in the gonads of *Astyanax mexicanus* treated with ACTH or cortisone. Low levels of lipid in the serum of females starved in the present study approximate serum lipid levels of starved male *Squalus* reported above (Sargent et al. 1971).

In mammals, which rely principally upon lipid metabolism during periods

of inanition, total serum lipid concentrations approximate those of freshly caught *Squalus* (*Homo*, 360–820 mg%; Harper 1969). During starvation, the turnover rate for serum fatty acids is under 8 min (Newsholme and Start 1973). This is in contrast to the situation in fasting elasmobranchs. Total serum lipids decline, and the turnover rate for serum fatty acids in starved *Squalus* is about 48 h (Sargent, Gatten, and McIntosh 1972).

Thus, it appears probable that circulating lipid components do not play a major role in the support of fasting elasmobranchs. Baldridge (1972) examined animals (*Carcharhinus milberti*, *Negaprion brevirostris*) starved in laboratory pens, and concluded that liver lipids were mobilized, but not in preference to tissue proteins. He suggested that lipids were conserved to meet buoyancy requirements. The normal liver of *Squalus acanthias* contains triglycerides, diacyl-glycerols, and wax esters (Sargent, Gatten, and McIntosh 1971.) The latter components are of low density and are presumably most important in buoyancy regulation. Malins and Barone (1970) placed weights on dogfish and found a significant increase in the ratio of diacyl-glycerols to triglycerides after 50 h of weight stress. They suggested that buoyancy was regulated by increasing the amount of low-density lipids in the liver. As Baldridge (1972) has observed, starvation would tend to increase underwater weight, since the denser tissues of the body, such as denticles, teeth, and calcified cartilage, are not metabolically available. Thus, while the mobilization of triglycerides and free fatty acids during starvation would provide some metabolic fuel, mechanisms may be present to conserve or even augment those lipids responsible for buoyancy. This has been suggested elsewhere by Sargent et al. (1972), following studies on the patterns of liver lipid synthesis in *Squalus*.

As in higher vertebrates, carbohydrate metabolism in elasmobranchs is subject to endocrine control. The hyperglycemia of depancreatized animals (*Mustelus*) was eliminated after pituitary extirpation (Dodd 1961), and ACTH administration to hypophysectomized animals elevated blood glucose (Grant and Banks 1967). The basic responses of serum glucose to insulin, glucagon, ACTH, corticoids, and catecholamines are similar in form to those of higher vertebrates (deRoos and deRoos 1972; Patent 1970).

Blood glucose levels are subject to a variety of influences, and variability is anticipated both between animals and, over time, in individuals. Normal serum glucose levels in elasmobranchs have been reported as ranging from 17–80 mg% (Denis 1922; Scott 1921; Patent 1970; deRoos and deRoos 1972). Patent (1970) sampled groups of freshly caught animals (*Squalus acanthias*) with blood glucose levels of 30 mg%, and felt that this reflected a poor nutritional state. Scott (1921) reported only "trace" amounts of glucose present in some animals held "until needed" in live cars; these animals generally appeared to be in poor condition.

In the present study, serum glucose levels declined during starvation. Decreases in glucose during fasting were also observed by deRoos and deRoos (1973) in *Squalus acanthias*, Kern (1966) in *Scyliorhinus canicula*, and Hartman et al. (1941) in rajids. However, Patent (1970) reported that blood glucose levels in *Squalus acanthias* remained stable during at least two weeks of starvation.

Glycogen reserves in the liver may not play a significant role in maintaining blood glucose during starvation. Patent (1970) reported that liver glycogen levels of freshly caught *Squalus acanthias* averaged 62.4 mg%. The liver of a 4-kg animal would approximate 400 g (Burger 1967*b*); thus liver glycogen reserves would total only about 250 mg (about 1000 calories).

Stimpson (1965) has suggested that the glycogen and lipid reserves of goldfish are made available solely under the influence of adrenaline, but not during starvation, when tissue proteins appear to be used preferentially. In elasmobranchs, liver glycogen has been shown to be unresponsive to corticoids and ACTH, though serum glucose levels were elevated (Patent 1970); administration of adrenaline decreased liver glycogen levels in addition to raising serum glucose levels (Grant and Banks 1967). Patent (1970) suggested that corticoids act through gluconeogenesis from liver lipids. However, deRoos and deRoos (1972) failed to find a significant decrease in liver lipids during cortisol-induced hyperglycemia, and suggested gluconeogenesis from protein precursors.

In the present study, serum protein levels declined steadily. The rapidity of this decline agrees with data of Cordier, Barnoud, and Brandon (1957), who found reductions in serum protein levels of *Scyliorhinus canicula*, starved over a two-week period, comparable to those in carp starved for six months. The increases in total body water (Baldridge 1972), intercellular fluid (present study, from histological interpretations), and rising serum potassium levels may also indicate protein catabolism and decreased plasma oncotic pressure. (The apparent muscular atrophy has been considered above.)

In elasmobranchs, urea levels are responsive to a variety of factors encountered in physiological studies, and thus are probably poor indicators of metabolic activities. As adrenaline administration increases urinary urea clearance some 15 times (Forster, Goldstein, and Rosen 1972), such factors as the stresses of capture and aggressive interactions in the pen, which influence the sympathetic nervous system, would depress serum urea levels. Moreover, the average water temperature over the experimental period (16°C) was close to the temperature at which urea clearance at the gills increases markedly (Boylan 1967: *Squalus acanthias*). And if the animal is gaining water, fluid shifts would affect the extracellular fluid concentration of a number of substances, urea included.

Metabolic Regulation

The regulation of secretion of the interrenal gland in elasmobranchs is mediated in a manner analagous to that of "higher" vertebrates. ACTH is present in the hypophysis (deRoos and deRoos 1964, 1967), and interrenal tissues respond to the presence of ACTH, or pituitary extracts, by an increase in steroid synthesis (Macchi and Rizzo 1962). The effects of pituitary extirpation, corticoid injection, and ACTH administration upon blood glucose levels have been noted above. Cortisol treatment decreases the secretory rate of the rectal gland (Chan and Phillips 1967) and in the present

study, rises in serum sodium and chloride were accompanied by interrenal hypertrophy and histological alterations in the rectal gland, which suggest a decrease in its secretory activity.

The chemical structure of the interrenal secretion is controversial In a series of papers (Idler and Truscott 1966; Idler and Truscott 1969; Grimm, O'Halloran, and Idler 1969; Truscott and Idler 1968; Truscott and Idler 1972; Idler, Freeman, and Truscott 1967; Idler 1969) the major steroid produced has been described as a unique form 1-α-OH-corticosterone. The interrenal organ of *Squalus acanthias* was reported to produce predominantly 1-α-OH-corticosterone, along with fractional amounts of corticosterone. However, Bern, deRoos, and Biglieri (1962) and Simpson and Wright (1970) reported little or no 1-α-OH-corticosterone and stated that the interrenal gland produced predominantly corticosterone and deoxycorticosterone.

As none of the aforementioned studies reported significant cortisol or cortisone production, the glucocorticoid competence of elasmobranch interrenal secretions may be questioned. The lack of extensive liver lipid mobilization, the decline in serum protein and glucose, and the preferential utilization of tissue proteins during starvation in elasmobranchs parallel the responses of other vertebrates to hyposecretion of glucocorticoids.

Although interrenal secretion probably decreased as death approached, it should be noted that both the alterations in the histological appearance of the rectal gland and hematopoeitic system and the rising serum sodium and chloride levels were consistent until the moment of death. Both cytological and metabolic changes appeared immediately after introduction to the pens, when the interrenals appeared most active; thus the lack of glucocorticoid activity was not due simply to interrenal insufficiency.

Extracts of elasmobranch interrenals have been shown to possess powerful mineralocorticoid activities, but no glucocorticoid properties, when administered to adrenalectomized rodents (Idler, Freeman, and Truscott 1967; Idler, O'Halloran, and Horne 1969). Both mineralocorticoid activity and a significant anti-inflammatory response accompanied the histological indications of interrenal secretion in the present study.

If we assume that interrenal secretions would produce a similar response in other vertebrates, the steroid must have a unique structure, since no adrenal steroid or combination found in higher vertebrates would produce high mineralocortoid , and anti-inflammatory activities in the absence of significant gluocorticoid activity (Frieden and Lipner 1969). It may be hypothesized that this hormone evolved in concert with the reliance upon liver lipids for buoyancy regulation; however, limiting the accessibility of lipids maintains the hydrodynamic efficiency of the animal but severely restricts its ability to tolerate prolonged inanition.

Figure 10 is a summary of the proposed interactions of factors affecting captive elasmobranchs. These have been defined as:

1. "Chronic stresses" associated with the conditions of confinement (such environmental variables as crowding, aggressive interaction, temperature, and starvation); these have been discussed in detail previously, and

PHYSIOLOGICAL TRAUMAS
(EXERTIONS ASSOCIATED WITH CAPTURE)

DEATH
(ACIDOSIS)

(c)

(b,d)

↑PH

(b)

(a,b)   ↑LACTIC   (d)   EXERTIONS ASSOCIATED
↓pH VIA   ACID   WITH CONFINEMENT
BUFFERS

(e)

↓OXYGEN   DEATH
CAPACITY OF   (HYPOXIA)
THE BLOOD

↓HEMATOCRIT   ↓SWIMMING
(f,g,h,p)   ACTIVITY (p)

↑HEMOLYSIS

↑K⁺
(p,f)

ALTERATIONS IN   ↑Fe⁺⁺
HEMATOPOIETIC   {↓
CENTERS (p)   ↓Cl⁻ (p)

↑Na⁺ (p)   ↓RECTAL GLAND
ANTI-INFLAMMATORY   ACTIVITY (p)
ACTIVITY

TISSUE
MINERALOCORTICOID   ↓SERUM   ↑CATABOLISM (p)
ACTIVITY (l)   PROTEINS (p)

↓GLUCOSE
↑TOTAL BODY   ↑INTERCELLULAR FLUID   (p,g,h)
WATER (m)   (p)
↑INTERRENAL   ↓TOTAL LIPIDS
SECRETION (p)   (SERUM) (p)
C(r)
↓CHOLESTEROL (p)
ACTH   STARVATION

DEATH
CHRONIC STRESSES   (DEPLETION)
(ASSOCIATED WITH CONFINEMENT)

a. PIIPER ET AL., 1972   g. BURGER, 1967   m. BALDRIDGE, 1972
b. PIIPER ET AL., 1969   h. ROBIN ET AL., 1966   n. FISHMAN, 1967
c. BLACK, 1958   i. CORDIER ET AL., 1957   o. PIIPER ET AL., 1970
d. MURDAUGH AND ROBBIN, 1967   j. deRoos AND deRoos, 1973   p. PRESENT STUDY
e. von BUDDENBROCK, 1936   k. KERN, 1966   q. SATCHELL, 1960
f. HARTMAN ET AL., 1941, 1944   l. IDLER, FREEMAN & TRUSCOTT, r. MACCHI & RIZZO, 1962
                                   1967

Figure 10 Hypothetical interaction of factors affecting survival of Elasmo-
branchs in activity.

2. Physiological traumas associated with capture and confinement.

These are not distinct in their overall effects, but each is associated with a
somewhat different complex of factors.

Sharks that struggle on a longline for a time often fail to survive for more
than a few hours, even with minimal blood loss and tissue damage sustained

in capture. Typically, these animals become immobile; eventually gill movements weaken and finally cease at death. A similar immobility and decrease in respiratory activity may be elicited in active elasmobranchs by lowering the oxygen content of the surrounding water (Fishman 1967; Piiper, Baumgarten, and Meyer 1970; Satchell 1960), and is considered a response to hypoxia.

Black (1958) reviewed literature on death caused by hyperactivity in fishes. He suggested that the likely cause of death involved an accumulation of large amounts of lactic acid produced by anaerobic glycolysis during exercise. In *Scyliorhinus canicula*, electric shock produced a fall in pH to approximately 7; minimum values were reached 2 h after treatment (Piiper and Baumgarten 1969). The pH values returned to normal 8 h later. Piiper, Meyer, and Drees (1972) reported that peak lactic acid levels of more than 200 mg% were observed in *Scyliorhinus canicula* up to 8 h after 25 min of activity. Presumably, the animals that survived this lactate load were capable of buffering the hydrogen ions that accompanied it. Lactate metabolism in captive *Squalus* is very slow (Robin, Murdaugh, and Millen 1966; Murdaugh, Robin, and Drewry 1965).

It may be significant that the livers of fasting rats (but not feeding rats) show an impaired ability to metabolize lactate in the absence of gluco-corticoids (Newsholme and Start 1973), considering the apparent lack of glucocorticoid activity in elasmobranch interrenal secretions discussed above. *Squalus acanthias* held in laboratory tanks have been reported to have higher levels of blood lactate than those assayed either at the time of capture or in dockside live cars. These lactate levels severely limited the animals' abilities to tolerate further lactic acid loading (Murdaugh and Robin 1967). Thus it appears that exertions like those associated with capture produce large quantities of lactic acid, which remains in the circulation and may be augmented with increasing time in captivity.

Besides reducing the adaptability of the blood buffer system, elevated levels of blood lactate are alleged to have other deleterious effects. Reduction in the oxygen binding capacity of the blood, deformation of red blood cells, and a tendency toward hemolysis were reported by von Buddenbrock (1936) in cod and flatfish. Erythrocytes of carp and suckers swelled and hemolyzed in the presence of lactate concentrations of 270 mg% (Black and Irving, 1938); this is within the range of values previously noted in stressed *Scyliorhinus canicula* (Piiper et al. 1972). Thus it is possible that elevated lactate levels in captive *Squalus* resulted in erythrocyte destruction.

It would be difficult to attribute the decline in hematocrit to a single factor, however. Starvation reduces hematocrit values in rajids (Hartman et al. 1941), cod (Kamra 1966), and eels (Sano 1962). Hematocrit also declines under sampling stress (Wood and Randall 1970; Tamura, Yasuda, and Fujiki 1962) or following interrenalectomy (Biedl 1913; rajids).

The decline in hematocrit was accompanied by hypertrophy and increased pigment content of the reticuloendothelial cells of the spleen. Oddly shaped red blood cells and intravascular hemolysis increased, while erythroblasts and hemocytoblasts formed an increasing proportion of the circulating cells.

Lower hematocrit values for captive animals have been reported in other studies. Burger (1967) mentions "wild plasma" as having hematocrit values of 17–32, while "live car plasma" had hematocrits ranging from 6 to 33. Lenfant and Johansen (1966) found hematocrits from 13 to 26 in aquarium-held *Squalus*, and Robin et al. (1966), mentioned that "healthy fish, in the experience of this laboratory, have hematocrits greater than 15%." Dawson (1933) reported that captive *Mustelus* had a greater percentage of erythroblasts than did wild animals (up to 33%).

Since 90% of the oxygen transport in *Squalus* occurs via the hemoglobin of the red blood cells (Lenfant and Johansen 1966), a reduction in hematocrit and an increased percentage of erythroblasts would necessarily decrease the oxygen-carrying capacity of the blood. The behavioral changes reported in the study· are comparable to those described for elasmobranchs subjected to hypoxic conditions over relatively brief periods (Fishman 1967; Piiper et al. 1970), and many other alterations observed in captive *Squalus* appear similar to those induced in other fishes by reductions in the oxygen content of the surrounding water.

It may be significant that species that adapt to confinement for long periods are adapted for life on or near the bottom. They can provide strong respiratory currents while motionless and are the species that accept food shortly after capture.

Catfish subjected to periods of low oxygen tension showed a loss in appetite and a cessation of digestive processes (Bouck and Ball 1965); elasmobranchs starved in captivity for weeks occasionally regurgitate, or display upon autopsy, pieces of food in the digestive tract undisturbed by digestive processes (Patent 1970; Martini, personal observations).

Blood glucose levels in captive animals occasionally rose to above normal values $(158 \pm 10$ mg%, $n = 9)$ shortly before the death of an animal. Hematocrit values for these animals averaged 10%. Denis (1922) and Scott (1921) described hypoxic hyperglycemia in elasmobranchs following their removal from the water or maintenance in aquaria with low oxygen concentrations, and tench subjected to suffocation by confinement showed a gradual increase in serum glucose to more than 300 mg% over a 2-day period (Bange-Barnoud 1965).

The relationship between the gradual reduction of oxygen and histological changes in the kidney and interrenal are unknown. In mammals, chronic hypoxia results in extensive kidney necrosis (Pitts 1966), but in fishes extreme interrenal disorganization has been described following acute stress (Rasquin and Rosenbloom 1954, *Astyanax mexicanus*; Weatherley 1963, *Perca fluyiatilis*).

Implications for the Researcher

More rigorous criteria should be applied to the maintenance and selection of elasmobranchs for use as experimental animals. Because of the similarity of trends observed in several species of elasmobranchs while starving in captivity, it would appear that this study has significance for those working with

selachians other than *Squalus acanthias*. Although the condition of the animals, methods of maintenance, or time in captivity are rarely noted, these may be necessary for evaluating experimental results. The changes described in this study are great enough to significantly affect almost every aspect of the animal's physiology. Alterations in kidney structure would affect any study of kidney function. Rising sodium, potassium, and chloride levels and changing urea levels would presumably affect transport rates of all excretory sites. Alterations in enzyme activity, lipid relationships, and intermediary metabolism would be expected in response to prolonged starvation. Changes in total body water and compartmentalization of fluids are indicated. The assessment of a shark's condition by "activity and color of the skin" (Reznikoff and Reznikoff 1934) or by random selection from a population of individuals of unknown history reduces the reliability of data collected.

The following recommendations are indicated.

1. Attempts should be made to minimize the trauma of capture, and animals should be brought aboard as quickly as possible. Prolonged struggling on a line, in gill nets, or in trawls may affect serum parameters, such as glucose levels, and blood loss may alter others, such as serum proteins.

2. As the population varies with regard to the nutritional state of individuals, the "ideal" subject would be one with known history prior to the start of an experimental procedure. Thus, adjustment to the conditions of confinement may be a prerequisite for accurate physiological and metabolic studies. Starvation appears to be a factor posing major problems, and acceptance of food may be necessary criterion for "adjustment." Patent (1970) reported an increase in liver glycogen following forced feeding, but experiments conducted upon animals fed ad libitum would more closely approximate the "normal" responses. It may be significant that Rall (1967) described conditions under which *Squalus acanthias* may be induced to feed. The key factors appear to be a large "volume of tank:size of animal" ratio, and a small number of animals held in the pen. A circular tank may also have been important, since animals swimming around the perimeter have an unrestricted swimming space.

Rall (1967) reported the water temperature as $13°$–$15°$C. Some disagreement exists regarding the temperature tolerance of *Squalus acanthias*. While Simpson and Ogden (1932) state that "dogfish" die if held at greater than $18°$C, references may be found describing *Squalus* held at $27°$C (Rasmussen 1972) or $23°$C (Sudak and Wilbur 1960). In a study of thermal tolerance in marine animals, Huntsman and Sparks (1924) reported that lethal temperatures for *Squalus* ranged from $28.5°$C to $29.1°$C (when exposed to a rate of increase in temperature of $1°$/5 min). Many of the animals used in the present study were caught on the surface when the water temperature was $17°$C, and Lauter, Brown, and Trams (1968) obtained *Squalus acanthias* for their studies by handline fishing when the water temperature was $17.5°$C. It is possible that elevated temperatures affect the animal's adjustment to captivity by altering the metabolic rate and available oxygen. Environmental

temperature and survival times differed significantly over the two experimental years reported in this study, yet the patterns of change observed in serum glucose, hematocrit, cholesterol, etc., did not.

3. If experiments are conducted on animals starved in live cars, the time held in captivity, water temperature, and hematocrit should be recorded.

In the present study, the survival of a shark was best predicted by considering alterations in hematocrit and serum proteins:

$$Y = 0.267\ X + 4.625Z - 5.71 \qquad\qquad r^2 = 0.74$$
$$p = 0.0001$$

where

Y = days until death
X = hematocrit value
Z = serum protein, gm%

However, the change in hematocrit alone was a reasonable predictor of survival time:

$$Y = 0.43\ X - 2.2 \qquad\qquad r^2 = 0.65$$
$$p = 0.001$$

The slope of this relationship would presumably change under different experimental conditions, but the hematocrit value at death ($\approx$5%) would still be useful as an "endpoint" for calculating survival time once the rate of change in hematocrit was experimentally determined.

If hematocrits were determined on freshly caught animals and redetermined for the same animals prior to their use as experimental subjects, a stable hematocrit could be taken as a favorable indication, while a sharp decline would indicate that physiological alterations were occurring. Since hematocrit can be conveniently determined with a minimum of equipment, it has a number of advantages as a diagnostic tool.

SUMMARY

1. *Squalus acanthias* were placed in live cars to assess the effects of confinement and starvation on this species.

2. Four experimental groups were used, and blood and tissue samples were taken at intervals throughout a period of confinement of up to 23 days. These were compared with samples from freshly caught animals and animals left undisturbed in the experimental pens.

3. Behavioral changes involved a gradual decrease in spontaneous swimming and a loss of equilibrium followed by immobility and death. Attempts to induce feeding were unsuccessful.

4. Physiological alterations took place immediately on introduction to experimental pens; they included increases in serum levels of sodium,

potassium, calcium, chloride, and iron. Decreases were observed in serum protein, total lipids, glucose, and cholesterol. Serum urea and total osmolarity were variable.

5. Atrophy of skeletal muscle was apparent in the starved animals.

6. A drastic reduction in hematocrit values occurred, associated with alterations in hematopoeitic tissues. Erythroblasts and hemocytoblasts formed up to 24% of the circulating red blood cells. Other alterations in the blood over the experimental periods included eosinophilia, lymphocytopenia, thrombocytopenia, and leukocytosis in the presence of immature erythrocytes, erythroblasts, and hemocytoblasts. Alterations in the spleen over the period of confinement included increased phagocytic activity of reticulo-endothelial cells, reticulo-endothelial hyperplasia, lymphocytopenia, and a progressive expansion of the red pulp.

7. The interrenal tissue evidence an increase in secretory activity. Nuclear enlargement and an increase in the vascularity of the gland were followed by a gradual depletion of lipid contents, and in some cases total disorganization of the glandular parenchyma. The lack of extensive liver lipid mobilization, the decline in serum protein and glucose, and the preferential utilization of tissue proteins during starvation parallel the responses of other vertebrates to hyposecretion of glucocorticoids. These results are consistent with the hypothesis that interrenal secretions of *Squalus* possess little or no glucocorticoid activities.

8. Histological alterations in the rectal gland were indicative of decreased secretory activity. Nephric tissue generally appeared normal histologically, but in animals sacrificed with low hematocrits, or sampled immediately after death, tubular degeneration was apparent.

9. No obvious changes were observed in the tissue of the endocrine pancreas; the exocrine pancreas displayed marked cellular atrophy. No consistent alterations were observed in the epithelium of the digestive tract.

10. When considered in combination with data of other authors on captive elasmobranchs, it is apparent that the alterations induced by fasting confinement are of sufficient magnitude to demand more rigorous criteria for the selection of experimental subjects.

11. Recommendations are presented for the capture, care, maintenance, and selection of elasmobranchs for use in experimental investigations.

12. In the light of this study, it is possible that many physiological and behavioral investigations of captive elasmobranchs should be reconsidered.

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VII RETROSPECT

THE OFFICE OF NAVAL RESEARCH
AND SHARK RESEARCH IN RETROSPECT

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The United States Navy has supported basic research on elasmobranchs for many years, largely through the Office of Naval Research. The main motive for this support is an obvious one: sharks have been a recognized hazard to human beings in the sea for centuries. A secondary, though in the long term perhaps more important, motive was that knowledge of the shark's biology and sensory capabilities might provide insight into many biological processes. Perhaps this realization became clear only when details about the remarkable sensory sensitivity of sharks began to emerge.

Because the main impetus for studying sharks is their danger to man, the history of naval shark research is tied closely to that of the Navy's standard shark repellent, "Shark Chaser." Before the Second World War, if the Navy had any official position on sharks, it was that they were largely harmless to uninjured, alert people in the water. This attitude is understandable since there was disagreement among scientists and naturalists as to how dangerous sharks really were to man. On the whole, most sharks were considered scavengers that might try to bite almost anything in the water and might be attracted to wounded or dead men but could be expected to leave healthy ones alone.

After the war started, shark incidents were reported from the front, especially in the Western Pacific. It was not clear, however, how serious the problem was, or even whether there was a problem. Reports from the front lines were censored, and the popular press often portrayed sharks as harmless. An article in *Collier's* magazine in 1944 was entitled "The Shark is a Sissy." Even now, it is still not clear how dangerous sharks actually were to men in the water. However, they were certainly a psychological threat, and the fear of sharks became a noticeable morale problem.

To deal with the problem, meetings were held in Washington in 1943. It seemed that the best way to handle this fear of sharks was to develop a shark deterrent, and it was suggested that the best possibility was a chemical repellent. It has since been pointed out that perhaps chemical shark repellents are not the most effective kind. For example, since a chemical repellent has never been used against such terrestrial predators as tigers or grizzly bears, why should one be useful against marine predators?

In the first place, there is no real evidence that the proper chemical repellent might not be effective to protect man against terrestrial predators. The most serious problem would probably be that man is sharply attuned to the same chemoreceptive system as these predators. For example, a vial of mercaptins such as a shunk produces would very likely repel predators, but to a human the smell would be unbearable. On the other hand, man is not attuned to water-borne chemical repellents or attractants the way aquatic animals are. Furthermore, evidence accumulating in recent years indicates that chemoreception may be much more important in the freshwater and oceanic realms, than in the terrestrial environment. All of this suggests that looking for a chemical shark repellent was not unreasonable and that ultimately, chemoreception may still provide the best means of repelling sharks.

When the assembled Navy, Coast Guard, Army Air Corps, and Merchant

Marine officers discussed how effective such a repellent would have to be, the consensus was that a deterrent that repelled sharks at least two times out of three, or 67% of the time, could be considered effective enough for distribution to personnel. However, one of the officers disagreed and wrote in a minority opinion that only repellents that worked nearly always should be issued. Curiously, no one present seemed to hold out for complete (100%) effectiveness, perhaps because of the realization that this would be an ideal that would be difficult, if not impossible, to attain.

To search for an effective chemical shark repellent, a research program was set up at the Naval Research Laboratory (Tuve 1963). The first clue was one that shark fishermen had long ago noticed; if a shark is caught on a fishing line, dies, and for some reason is not retrieved before decomposition sets in, then further fishing for sharks in that vicinity is useless. In other words, rotting shark meat appeared to act as a shark repellent, or at least a feeding inhibitor. Tests conducted in a chemical laboratory on samples of rotting shark flesh indicated that the most abundant chemical byproduct was ammonium acetate.

From other preliminary screening tests on a variety of chemicals at the Woods Hole Oceanographic Institution, maleic acid and copper sulfate showed some promise in inhibiting feeding in dogfish sharks. Maleic acid was subsequently eliminated, and, because of instability problems with ammonium acetate, the acetate portion was combined with the copper portion of the copper sulfate. Consequently, copper acetate was tested as the active ingredient. However, copper acetate was virtually invisible when dissolved, so a black pigment, nigrosine dye, was added in later tests to permit humans to see the chemical cloud in the water. It was added mainly for its psychological effect, since tests showed that the nigrosine dye diffuses at a different rate than the copper acetate. In any case, the mixture of 80% nigrosine dye and 20% copper acetate proved effective, sometimes nearly 100% effective, in keeping sharks from feeding, even when they were already actively feeding on trash fish shoveled off the deck of a shrimp boat.

Tuve (1963) and Gilbert and Springer (1963), in their detailed reviews of the repellent research program, discussed the results of the three sets of field tests conducted off northern Peru, Biloxi, Mississippi, and Mayport, Florida, noting that five kinds of sharks were listed in the Biloxi tests—blacktip, sharpnose, hammerhead, lemon, and tiger sharks. Few addditional species were involved in the Florida and Peru tests. Thus, many species of dangerous sharks were not tested, nor were tests conducted in nonfeeding situations. However, the tests that were conducted indicated an overall effectiveness of more than 67% for the mixture of nigrosine dye and copper acetate. Consequently, these chemicals, mixed in a cake of water-soluble wax that dissolved over a 3-h period, became the standard issue "Shark Chaser." Whether or not it effectively repelled sharks under combat conditions, it was certainly effective psychologically and helped morale, which was probably the most important problem at that time. In fact, the psychological aspect—the fear of sharks—continues to be one of the most serious problems.

Given this kind of background, it shouldn't have been surprising that after

the war reports of cases in which "Shark Chaser" didn't work were heard; sharks were even seen to have bitten cakes of it and swum off with black clouds streaming from their gill slits. After all, "Shark Chaser" had never been tested on all species of dangerous sharks under different conditions or in different environments. Furthermore, it had never been 100% effective in the limited tests that had been conducted, nor was it ever expected to be 100% effective. However, as the reports came in, the Navy took action to find out the full story about sharks and "Shark Chaser."

At that time, research on some problems in biological oceanography was handled through the Ecology Section of the Human Ecology Branch in the Medical Sciences Division of the Office of Naval Research, with Dr. Sidney R. Galler acting as a consultant in ecology. In 1950, the Ecology Section of the Human Ecology Branch was combined with the Biophysics Branch to form the Biology Branch, with Dr. Galler as Program Director.

Biology branch work consisted of four programs. The first two were major programs, one on survival under extreme environmental and geographic conditions with emphasis on polar regions, and the other on the impact on the human body of mechanical and other stresses such as vibrations and shock. The third program was on biological orientation, and the fourth was the hydrobiology program, which included the problem of survival at sea. One component of this dealt with protecting personnel against venomous, carnivorous, and toxic organisms. This component was a direct outgrowth of the Navy's experience in the western Pacific Ocean during World War II, where some individuals died from eating poisonous fish, some had been injured by such venomous organisms as stonefish, and some had been attacked by sharks. This was the beginning, in a semiformal sense, of renewed Navy interest in protecting personnel against sharks. The dangerous and noxious marine organisms research program then became part of the Oceanic Biology Program when it evolved from the Biology Branch with the establishment of the Ocean Science and Technology Division. The Oceanic Biology Program included the nonhuman-related biology from the old Biology Branch. The most recent change came about when the entire Ocean Science and Technology Division was transferred from the Office of Naval Research headquarters outside Washington, D.C., and made part of the Naval Ocean Research and Development Activity (NORDA), located at the National Space Technology Laboratories in Mississippi but still a part of the Office of Naval Research.

Formal renewal of the Navy's interest in shark-related problems can perhaps be traced to a conference on elasmobranchs sponsored by the American Institute of Biological Sciences (AIBS) and supported by the Office of Naval Research and Navy Bureau of Aeronautics. At the conference, entitled "Basic Research Approaches to the Development of Shark Repellents," held at Tulane University April 8 to 11, 1958, 34 scientists reviewed what was then known about the biology and behavior of dangerous sharks. One result was the establishment of the AIBS Shark Research Panel, and a second was the publication of *Sharks and Survival*, edited by Perry Gilbert (1963). *Sharks and Survival* was a compendium of

what was known about sharks and their hazards. As such, it is a landmark in elasmobranch biology.

The Shark Research Panel, under the chairmanship of Gilbert, became an adjunct of the AIBS Hydrobiology Committee and met 31 times from 1958 to 1970 (Appendix) to review the status of elasmobranch research.

One of the first recommendations of the Shark Research Panel was that a comprehensive program of basic studies on the taxonomy, behavior, and functional anatomy of dangerous sharks was essential to understanding the shark hazard. This recommendation has guided the shark research program of the ONR Oceanic Biology Branch through the years.

The early research efforts consisted of several projects to lay the necessary groundwork. The first and longest was establishment of the International Shark Attack File under the sponsorship of the AIBS, Cornell University, the Smithsonian Institution, and ONR. While active data collection for this project ended in 1970, computer-assisted analysis of the data took time to complete. The final report represents the most complete analysis of shark incidents ever undertaken (Baldridge 1974). Since 1970, reports of shark incidents have been added to the Shark Attack File only sporadically. Though the basic research phase of this project has been completed, and continued collection of information is really only record keeping, there is no doubt that it is important; efforts are being made to continue the project on a more active basis.

Other projects that might be considered as laying groundwork included research on the taxonomy of several of the poorly known groups of sharks, such as the hammerhead and requiem sharks, and construction of facilities for maintaining sharks and conducting experiments on them under controlled but seminatural conditions. Examples of such facilities were the shark pens at the Rosenstiel School of Marine and Atmospheric Sciences of the University of Miami, the shark pens at the Lerner Marine Laboratory of the American Museum of Natural History in the Bahamas, and the pools at the former site of the Mote Marine Laboratory. Another example of a specialized, innovative facility that led to an increased understanding of the behavior of some species of sharks (as well as other kinds of fish) was the remotely operated underwater television camera at the Lerner Marine Laboratory in the Bahamas. Unfortunately, these facilities are no longer used for elasmobranch research.

Elasmobranch research grew with a number of projects supported by ONR, and as a result of ONR's leadership, by the navies of other countries. For example, the first Inter-American Naval Marine Biological Conference, held in Puerto Rico in 1966, brought together representatives of the research arms of most of the Latin American navies. One item of mutual interest for discussion at the conference was the problem of personnel protection under emergency survival conditions, including the hazard of shark attacks. Other conferences dealing with elasmobranch research were sponsored wholly or in part by ONR as the shark research program grew. From January 30 to February 4, 1966, a conference entitled "Current Investigations Dealing with Elasmobranch Biology" was held at the Lerner Marine Laboratory at Bimini,

Bahamas. It resulted in the publication of *Sharks, Skates and Rays*, edited by Perry W. Gilbert, Robert F. Mathewson, and David P. Rall (1967). In November 1975 a conference entitled "Sharks and Man: A Perspective" was held at Orlando, Florida, under the auspices of the Florida Sea Grant Program and cosponsored by the Florida Department of Natural Resources, National Marine Fisheries Service, and ONR. Then, in May 1976, a symposium on elasmobranch biology, partly sponsored by ONR, was held as part of the annual meeting of the American Society of Zoologists. The papers appeared in a dedicated volume of the *American Zoologist* (Spring 1977, Vol. 17, No. 2).

The shark research program has evolved since the 1958 New Orleans meeting but has remained faithful to the recommendations of the Shark Panel that grew out of that meeting. As knowledge of sharks has increased, such research areas as taxonomy have been deemphasized.

However, a major area of continuing investigation is shark behavior, since progress here has been not nearly as great. While several recent studies on the behavior of individual species indicate that shark behavior, like that of other animals, can be quite predictable, understanding their behavior depends on understanding their sensory capabilities. Consequently, the shark research program over the past several years has emphasized the sensory biology of sharks—their vision, hearing, chemoreception and sensitivity to weak electric fields. From these studies has come a greater appreciation of why sharks have been such successful predators for so long. These and other findings were discussed at a 1974 conference on shark research supported by ONR and summarized in the report of the conference (Zahuranec 1975).

An understanding of the remarkable adaptions of sharks has also made clearer why "Shark Chaser" was not as effective as desired. As a result, "Shark Chaser" is no longer issued as survival gear to Air Force and Navy airmen. Ultimately, it will be removed from the standard military specification lists. In the Navy, the organization that has charge of survival at sea for airmen is the Naval Air Systems Command (NAVAIR). The recent emphasis in NAVAIR seems to be toward keeping personnel out of the water in one-man life rafts as a means of reducing the shark hazard. This not only alleviates much of the threat of shark attacks but also greatly reduces heat loss.

However, the most important question the Navy must next resolve is "How much danger do sharks actually pose to Navy personnel?" The question has been asked many times in many forms for many years, and has never been answered adequately. The usual brief answer is "Sharks are more important psychologically than physically." That is, the fear of sharks is much more important than the actual damage they do to humans. Probably the most complete attempt to quantify the direct danger that sharks posed to military personnel was a *post facto* analysis of World War II records conducted by George Llano (1955). He found that out of nearly 2000 personnel who found themselves in the sea, in only about 10 cases were sharks involved in injuries or deaths. Of course, Llano pointed out, there might be no report at all of those incidents most successful from the shark's

standpoint, i.e., those in which no traces of the human remained. In addition, the records were spotty at best; reports of the cause of death or injury were very imprecise and left a great deal to be desired. This was due, at least in part, to psychological pressure on armed forces personnel to list the cause of injury or death as other than due to sharks, to downplay the shark danger and help boost morale.

As difficult as it is to obtain accurate quantitative information about the direct shark hazard to personnel, it is more difficult to obtain accurate information on the psychological aspects of the shark threat to man in the sea. A workshop of military personnel held in May 1976 to discuss the impact of shark hazards on Navy and Marine Corps operations recommended obtaining such information. In particular, the workshop recommended that information be gathered on incidents involving divers in which sharks directly affected military operations (Zahuranec 1976). As difficult as these data are to obtain, it is imperative that the best possible attempt be made. Only when there is sufficient information of this nature can a shark research program deal accurately with the more acute aspects of the problem. In the meantime, the Navy's shark research program will continue to provide information on shark behavior as well as other aspects of shark biology, since such knowledge can be important to Navy as well as non-Navy personnel. Indeed, probably as long as man is in the sea, for whatever reason, sharks will continue to be dangerous adversaries, but ones whose importance is out of all proportion to their numbers.

ACKNOWLEDGMENTS

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APPENDIX

SHARK RESEARCH PANEL MEETINGS

1.	June 25, 1958	Office of Leonard P. Schultz
2.	Dec. 5–7, 1958	Lerner Marine Laboratory, Bimini (a joint meeting with AIBS Hydrobiology Committee)
3.	Dec. 29, 1958	Shoreham Hotel, Washington, D.C. (during AAAS Meetings)
4.	Jan. 14, 1959	AIBS Headquarters, Washington, D.C.
5.	Feb. 25, 1959	AIBS Headquarters, Washington, D.C.
6.	Apr. 23, 1959	AIBS Headquarters, Washington, D.C.
7.	Sept. 24, 1959	AIBS Headquarters, Washington, D.C.
8.	Jan. 29, 1960	ONR and AIBS Headquarters, Washington, D.C.
9.	Mar. 4, 1960	DuPont Plaza Hotel and AIBS Headquarters, Washington, D.C.
10.	Sept. 9, 1960	AIBS Headquarters, Washington, D.C.
11.	Jan. 27, 1961	American Museum of Natural History, New York, N.Y.
12.	Mar. 27–30, 1961	Lerner Marine Laboratory, Bimini
13.	Aug. 25, 1961	University of Hawaii, Honolulu (Conference on Anti-Shark Measures held in conjunction with the Tenth Pacific Science Congress)
14.	Dec. 13–16, 1961	Lerner Marine Laboratory, Bimini
15.	Feb. 2, 1962	AIBS Headquarters, Washington, D.C.
16.	Apr. 20, 1962	AIBS Headquarters, Washington, D.C.
17.	Nov. 28, 1962	AIBS Headquarters, Washington, D.C.
18.	Jan. 30, 1963	DuPont Plaza Hotel, Washington, D.C. (executive meeting of panel)
19.	July 5, 1963	Ben Franklin Hotel, Philadelphia, Pa. (open meeting with Underwater Society of America Annual Convention)
20.	Mar. 13, 1964	Scripps Institution of Oceanography, La Jolla, Calif.
21.	Nov. 19, 1964	Marriott Motor Hotel, Key Bridge, Arlington, Va.
22.	Mar. 3–4, 1965	University of Puerto Rico, Mayaguez, P.R.
23.	Nov. 9, 1965	Gramercy Inn, Washington, D.C.

24. Feb. 3, 1966   Lerner Marine Laboratory, Bimini
25. Feb. 17, 1967   California Academy of Sciences, San Francisco, Calif.
26. Oct. 26, 1967   AIBS Headquarters, Washington, D.C.
27. Apr. 25–26, 1968   Cosmos Club; Watergate Hotel; Smithsonian Institution, Washington, D.C.
28. Nov. 14–15, 1968   Mote Marine Laboratory, Sarasota, Fla.
29. Nov. 13–14, 1969   California Academy of Sciences, San Francisco, Calif.
30. Jan. 17–18, 1970   Honolulu, Hawaii
31. Mar. 24–25, 1970   New Orleans, Louisiana

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